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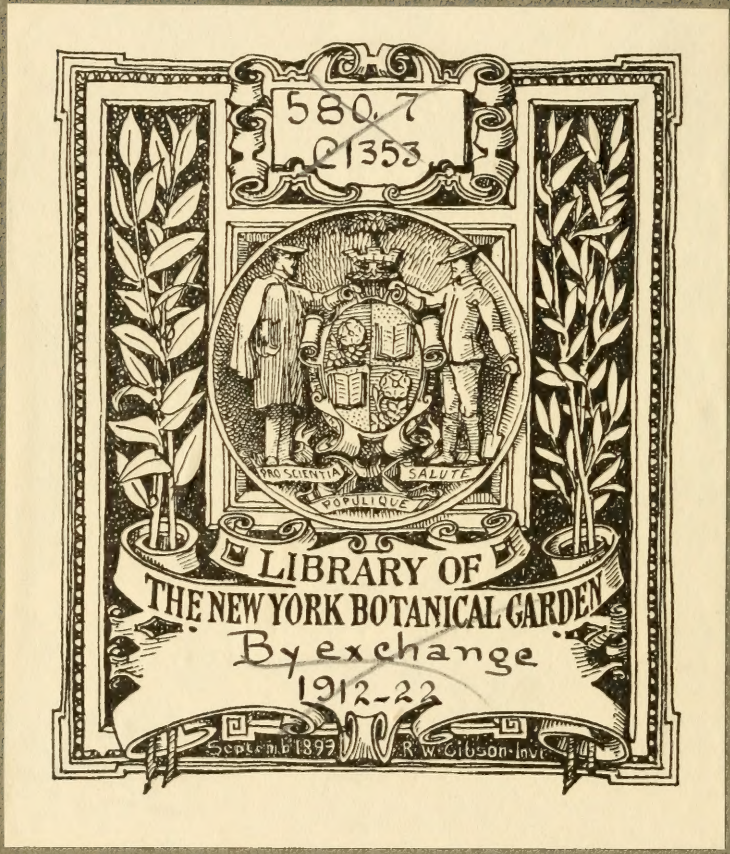
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University of California Publications in BOTANY

VOLUME V
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EDITOR
WILLIAM ALBERT SETCHELL

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In addition to the seventeen papers included in this volume, the following articles based upon results obtained in this same series of investigations have been published elsewhere.

Variation of Flower Size in *Nicotiana*. T. H. Goodspeed and R. E. Clausen. Proc. Nat. Acad. Sci., vol. 1, pp. 333–438, 1915.

Parthenocarpy and Parthenogenesis in *Nicotiana*. T. H. Goodspeed. *Ibid.*, pp. 341–346, 1915.

Factors Influencing Flower-Size in *Nicotiana* with Special Reference to Questions of Inheritance. T. H. Goodspeed and R. E. Clausen. Amer. Jour. Botany, vol. 2, pp. 332–374, 1915.

Hereditary Reaction-System Relations—An Extension of Mendelian Concepts. R. E. Clausen and T. H. Goodspeed. Proc. Nat. Acad. Sci., vol. 2, pp. 240-244, 1916.

Mendelian Factor Differences versus Reaction-System Contrasts in Heredity. T. H. Goodspeed and R. E. Clausen. Amer. Nat., vol. 51, pp. 31–46 and 92–101, 1916.

A Preliminary Note on the Results of Crossing Certain Varieties of *Nicotiana Tabacum*. W. A. Setchell, T. H. Goodspeed, and R. E. Clausen. Proc. Nat. Acad. Sci., vol. 7, pp. 50–56, 1921.

Inheritance in *Nicotiana Tabacum*. II, The Existence of Genetically Distinct Red-Flowering Varieties. R. E. Clausen and T. H. Goodspeed. Amer. Nat., vol. 55, pp. 328–334, 1921.

Volume 11 of the *University of California Publications in Botany* will contain further articles dealing with the tobacco investigations.

CONTENTS

		PAGES
Acknowledgment		i
No. 1.	Studies in *Nicotiana*, I, by William Albert Setchell	1–86
No. 2.	Quantitative Studies of Inheritance in *Nicotiana* Hybrids, by Thomas Harper Goodspeed	87–168
No. 3.	Quantitative Studies of Inheritance in *Nicotiana* Hybrids, II, by Thomas Harper Goodspeed	169–188
No. 4.	On the Partial Sterility of *Nicotiana* Hybrids Made with *N. sylvestris* as a Parent, by Thomas Harper Goodspeed	189–198
No. 5.	Notes on the Germination of Tobacco Seed, by Thomas Harper Goodspeed	199–222
No. 6.	Quantitative Studies of Inheritance in *Nicotiana* Hybrids, III, by Thomas Harper Goodspeed	223–231
No. 7.	Notes on the Germination of Tobacco Seed, II, by Thomas Harper Goodspeed	233–248
No. 8.	Parthenogenesis, Parthenocarpy, and Phenospermy in *Nicotiana*, by Thomas Harper Goodspeed	249–272
No. 9.	On the Partial Sterility of *Nicotiana* Hybrids Made with *N. sylvestris* as a Parent, II, by T. H. Goodspeed and A. H. Ayres	273–292
No. 10.	On the Partial Sterility of *Nicotiana* Hybrids Made with *N. sylvestris* as a Parent, III, by T. H. Goodspeed and J. N. Kendall	293–299
No. 11.	The Nature of the F_1 Species Hybrids between *Nicotiana sylvestris* and Varieties of *Nicotiana Tabacum*, by T. H. Goodspeed and R. E. Clausen	301–346
No. 12.	Abscission of Flowers and Fruits in the Solanaceae, with Special Reference to *Nicotiana*, by John N. Kendall	347–428
No. 13.	Controlled Pollination in *Nicotiana*, by Thomas Harper Goodspeed and Pirie Davidson	429–434
No. 14.	An Apparatus for Flower Measurement, by T. H. Goodspeed and R. E. Clausen	435–437
No. 15.	Note on the Effects of Illuminating Gas and its Constituents in Causing Abscission of Flowers in *Nicotiana* and *Citrus*, by T. H. Goodspeed, J. M. McGee, and R. W. Hodgson	439–450
No. 16.	Notes on the Germination of Tobacco Seed, III, by T. H. Goodspeed	451–455
No. 17.	Inheritance in *Nicotiana Tabacum*, I, A Report on the Crossing of Certain Varieties, by William Albert Setchell, Thomas Harper Goodspeed, and Roy Elwood Clausen	457–582

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STUDIES IN NICOTIANA. I.

BY

WILLIAM ALBERT SETCHELL

In 1906, as a consequence of a certain interest in the history of the origin and spread of the use of tobacco, I began to cultivate such species of *Nicotiana,* both native and horticultural, as I could obtain seed of, in the Botanical Garden of the University of California. In all a total of 103 packets were sown that year, and from 75 packets good plants were obtained. Of many of these, seed was produced under bag and for the most part this "pure seed" was from a single plant. Since 1906 the sowings have been continued each year, from seed of the previous year, as well as from seed from new sources, until finally a very considerable assortment of species and varieties have been grown. Gradually the number of plants have been reduced and selected to a certain number to serve as a stock for breeding. It is my intention in the present volume to publish the results of the experience of myself and others, in connection with the cultivation and experimentation with this stock of *Nicotiana* in the Botanical Garden of the University of California.

The expenses of these investigations have been borne partly by the funds granted by the President and Regents of the University of California for the up-keep of the Botanical Garden and partially by certain allotments from that portion of the Adams Fund of the United States Department of Agriculture granted to the Agricultural Experiment Station of the University of California. Through these means the work on the *Nicotiana* species, detailed in this volume, has been carried on.

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The sources of supply for seeds have been collectors and botanical gardens. From various collectors, seeds of wild species have been obtained, particularly of the Pacific Coast and of Texas. From the botanical gardens of Europe and America I have been able to obtain seeds of most of the cultivated species, and from the various divisions of the Bureau of Plant Industry I have been able to obtain seeds of many of the cultivated varieties and forms of *Nicotiana Tabacum.*

At first the study was largely systematic and morphological, with the entirely and naturally to be expected result that it was found that very considerable confusion existed in the naming of the different plants. Some two or three years were devoted to growing almost everything that could be obtained and attempting to straighten out the nomenclature, which has been a matter of no little difficulty. The only recent revision is that of Comes (1899) and this has been used, so far as it clearly applies, in unravelling the tangle of names current in trade and in the botanical gardens.

After careful growing and selecting, about seventeen species as generally recognized, remain, together with some well-marked varieties. It seems best to enumerate and discuss these to some extent, in order that a basis may exist for more exact knowledge of the work in the following pages. I hope, also, to be able to return to these later and publish a critical discussion of them.

Comes in his "Monographie du genre Nicotiana" (1899), divides the genus into four sections, viz., I, *Tabacum*; II, *Rustica;* III, *Petunioides,* and IV, *Polidiclia.* This classification is that of G. Don (1838) as well as of Dunal (1852). For reasons which will be discussed in a later paper, it seems that IV, viz., *Polidiclia,* cannot be separated from III, viz., *Petunioides.* Consequently I have retained I, II, and III, and will discuss the stock of species and varieties collected in the Botanical Garden of the University of California under these three sections. (At the very time of writing this, I receive East's paper (1912) entitled "A Study of Hybrids between *Nicotiana Bigelovii* and N. *quadrivalvis.*" He also gives very convincing reasons for combining the sections *Petunioides* and *Polidiclia*).

Section I. Tabacum G. Don

Nicotiana Tabacum L.

Under this section there is usually included only one species, viz., *Nicotiana Tabacum* L. It seems to include all those species of *Nicotiana* in which the corolla is of various shades of red or occasionally white (albino?), infundibuliform, with the throat somewhat to much inflated, and with the limb patent. The flowers are in panicled corymbs. Under the single species *N. Tabacum* is grouped a most varied assemblage of varieties, forms, and suspected hybrids as ever were brought together under one specific name. Comes (1895, 1899, and 1905) has attempted to arrange the various forms under some six varieties and also under various supposed combinations of hybrid origin. The result has been to bring some order out of chaos and to point out certain experimental possibilities. Anastasia (1906) has also concerned himself with an inquiry into the typical varieties of *Nicotiana Tabacum*. He differs somewhat in his ideas from Comes. Howard and Howard (1910) have attempted to arrange and illustrate the types of Indian tobaccos constant in their characters, and Hasselbring (1912) has just discussed the types of *N. Tabacum* grown in Cuba, showing that they remain true to type when grown also in Michigan.

In these various papers it is evident that all the variety of plants to be referred under *N. Tabacum* in its broad and comprehensive use fall under seeming combinations of a few types. The further question as to the origin of these combinations has, as yet, been merely suggested but not proven. Probably there will be no exact agreement, for a while, as to just what the simplest expressions of the fundamental types are, nor can it be settled except by continued and extended experimentation, if it can ever be settled at all. Comes has selected and described six typical varieties and referred all others as combinations of two or more of these. Anastasia thinks that there are only four. I have tentatively selected five as seemingly fundamental. These three sets thus selected are not coincident for the greater part.

Since it is my intention to discuss this matter later and since, at present, it is desirable only to enumerate and make reasonably plain what plants have been used in the work carried on in the Botanical Garden of the University of California (U. C. B. G.), I shall content myself with giving a list with descriptive remarks of the types which have appealed to me as being possibly fundamental or of other interest in experimentation. In connection with each I shall use the number by which it has been designated in the U. C. B. G.

"Brazilian"

U. C. B. G. $\frac{71}{05}$.—The seed of this number was received under the name of "Choice Brazilian American" from the United States Department of Agriculture in 1905. Its habit and general characters are well shown in the photograph reproduced in plate 1. It is a tall plant, averaging about six feet in height, producing laterals in succession above, but barely being overtopped by them. The leaves are long, broad, and decidedly cucullate at the tip. They are thin and silky in texture, being minutely glandular-pubescent. The corolla is pink, tubular below gradually and moderately infundibuliform above, being more swollen than $\frac{68}{07}$, but less so than $\frac{72}{05}$, about equally so with those of $\frac{78}{05}$. Altogether the plant is very near to a plant grown in the U. C. B. G. from seed sent by Professor Dr. O. Comes, labelled as being of his *Nicotiana Tabacum* var. *brasiliensis*. It does not seem to answer exactly to his figure of that variety (1899, VI) nor exactly to that of *N. Tabacum* var. *havanensis* (*loc. cit.* VII). In his later work (1905, p. 81, fig. 27) Comes figures a plant which he designates as "Bahia" and which he considers as a combination of his varieties *brasiliensis* and *havanensis* which is close to our plant, but not exactly of the same inflorescence at least. The plant designated by Anastasia (1906, opp. p. 102) as *N. Tabacum* var. *brasiliensis* is very nearly the same as ours. It seems therefore, that we may designate the plants bred from $\frac{71}{05}$ U. C. B. G. seed as "Brazilian." Its exact characters and relation to other "Brazilian" combinations as well as "Havana" combinations will be considered when certain experiments involv-

ing it as one of the factors are discussed in a paper which it is hoped may be published later.

"Cavala"

U. C. B. G. $\frac{72}{05}$.—The seed from which the plants were raised and continued under this number in our botanical garden was obtained from the United States Department of Agriculture. They were labelled "Cavala Tobacco" and were No. 11497 of the U. S. D. A., obtained from Turkey. The habit and general characters of the plant are well represented in the photograph reproduced on plate 2. It is a tall plant with upper and middle laterals which more or less overtop the original panicle. The leaves are short, compared with those of nearly all the other members of the *N. Tabacum*-group, peculiarly and more decidedly rugose on the upper surface as well as velvety, shaped more like those of $\frac{22}{07}$ (*Nicotiana Tabacum* var. *macrophylla* Comes) but more tapering towards the base and long and narrowly decurrent. The flowers are also nearer to those of $\frac{22}{07}$ than to the others. In color, however, they are pink. The lobes are broad and rather shallow, but they are tipped with a short recurved point. The tube is slender below but is stout and broadly infundibuliform above.

This plant is not to be identified with any of the typical varieties of either Comes or Anastasia. Nor do I identify it with any of the cultivated varieties figured by them. I shall simply call it *Cavala* and discuss its position and influence in breeding, later. The texture of the surfaces of the leaf and the shape and decurrence of the leaf make it a desirable plant in crossing.

"Maryland"

U. C. B. G. $\frac{78}{05}$.—The seed from which the stock of plants designated by this number has been obtained was distributed by the United States Department of Agriculture in 1905, and designated as "Maryland," with the identifying number "205–20–7." In habit, as well as other characters, it is decidedly different from the two varieties just described. It is of some-

what lower stature and the spreading leaves at the base give it a sort of pyramidal (or conical) shape (cf. plate 3). The leaves are long, broad in the middle and tapering very rapidly to each end. At the apex is a fairly long point curved to one side, while the base is narrow, to expand at the junction of the stem into two broad clasping auricles. The panicle is ample as compared with that of other members of this section. The flowers are very light pink, with slender tube and infundibulum and with the limb broadly but deeply lobed. The lobes have slender incurved points. It would be classed by both Comes and Anastasia as a combination form under *Nicotiana Tabacum* var. *virginica*. It comes near to the plant figured by Anastasia (1906, opp. p. 30) and by Comes (1899, pl. V) but is not identical with either. It seems best to call it "Maryland." The original *Nicotiana virginica* Ag. (1819, p. 18), as represented by the type-specimen in the Herbarium of the University of Lund, is of the same general type, as is also the type of *Nicotiana Tabacum* L. of Linnaeus's herbarium in London. The type-specimen of *N. fruticosa* in the Linnaean Herbarium seems also to belong here but may possibly, however, represent the plant referred by Anastasia (cf. above under U. C. B. G. $\frac{71}{05}$) to var. *brasiliensis*. The plant in Hb. Agardh is decidedly narrow-leaved and may be near to what Comes (1899, p. 10, pl. IV) has called var. *lancifolia*. From what could be seen of the flower, it seemed to be broader-lobed than that represented by Comes for this latter variety.

Nicotiana Tabacum var. **calycina**

U. C. B. G. $\frac{110}{05}$.—This is the plant known in botanical gardens as *Nicotiana Tabacum* var. *calycina*. The seed was received from the Botanic Garden of the University of Cambridge in 1905. It has remained constant in its peculiarities, ever since, under conditions of pure-line breeding. In habit, it is peculiar, as shown in the photograph reproduced in plate 4. The lower laterals soon come to equal the main axis or even slightly to overtop it, so as to obscure it as a main axis. The leaves are large, approaching in shape the members of the *virginica*-group

(such as U. C. B. G. $\frac{78}{05}$). The lower leaves are broader (proportionally) in the middle and taper more abruptly to each end than do those of the *Maryland* (U. C. B. G. $\frac{78}{05}$). The auricles at the base are hardly discernible in the *calycina,* while they are decidedly pronounced in the *Maryland.* While this plant is distinct in habit and leaf, it is still more characteristic in its flower. The flower is double of the "hose-in-hose" pattern. The calyx is more or less petaloid and colored bright pink or light red, as is also the corolla. Sometimes the whole calyx is petaloid in color (whitish tube, pink above and deep pink limb) or it may have some green in it, usually irregularly distributed. Both calyx and corolla are split on one side, in most cases, and even to the very base. Both are deciduous, leaving the capsule naked. The lobes of the limb of both calyx and corolla are broadly but deeply lobed and the lobes have long laterally curved points. The capsule is more oblong than that of other members of the *Tabacum*-group. The inflorescence is more compact than that of other members of the *Tabacum*-group with the exception of the members of the group surrounding *N. angustifolia* (*N. Tabacum* var. *fruticosa* Comes, not Hook., U. C. B. G. $\frac{68}{07}$). In fact, this plant (U. C. B. G. $\frac{110}{05}$) combines characters of the *angustifolia*- and of the *virginica*-sections with its own peculiar teratological features. This plant will be referred to as *Nicotiana Tabacum* var. *calycina,* or simply as *calycina.*

"White Tobacco"

U. C. B. G. $\frac{30}{06}$.—A plant of the *Tabacum*-group with cream-white flowers has been bred in our botanical garden for several years, and in the pure-line cultures has retained its color and other characters perfectly. It came from seed distributed from the Missouri Botanical Gardens in 1905. An inquiry directed to Director William Trelease in 1910 as to its source, brought out the information that the original plants were found by him in Mexico, growing "in that interesting little village, Maltrata, at the foot of the first descent from the table-land down toward Vera Cruz on the Orizaba side." He

says further: "I found the ordinary pink-flowered form and the white one growing as wayside weeds and gathered a considerable quantity of the white form because of its striking appearance. From the occurrence of the plants I should suppose that there was every reason to anticipate crossing of the two forms but, so far as I knew of it, all of the seedlings that we raised from the white seed bred true." Our experience has been that it breeds true when protected by bag, but one year, seed which had been taken from an unprotected plant gave a pink-flowered form whose bagged seed, in turn, gave a considerable variety of colors, stature, etc., in the plants raised from them.

The *White Tobacco* as we have called it, is a tall plant, up to six feet high and over, of simple habit, only the upper flowering laterals developing. It is well shown in the photograph reproduced in plate 5. The leaves are much like those of *N. Tabacum* var. *macrophylla* Comes (U. C. B. G. $\overset{22}{\overline{07}}$) except that they are more rounded at the middle, have a decidedly prolonged point, and are rugose and downy above. The flowers are like those of U. C. B. G. $\overset{22}{\overline{07}}$ except in color.

This is a most interesting plant. It has the habit more of the *Cavala* (U. C. B. G. $\overset{75}{\overline{05}}$) of which it has also the peculiarities of the surface of the leaf (both rugosity and downiness). It has, however, the general leaf-shape and flower-shape of U. C. B. G. $\overset{22}{\overline{07}}$ (*N. Tabacum* var. *macrophylla* Comes), and these are combined with an apparently albino character in the flower. It is a poor seeder, a characteristic pointing toward a possible hybrid origin. The seed is apt to be both comparatively scanty and of poor germinating power.

Nicotiana Tabacum var. macrophylla

U. C. B. G. $\overset{22}{\overline{07}}$.—This is the *Nicotiana Tabacum* var. *macrophylla* of Comes and was grown from seed kindly supplied by Professor Comes from his plants at Portici near Naples. It has the essential characteristics described and figured by him for this variety (cf. Comes, 1899, p. 18, pl. VIII), but does not

correspond perfectly to his figure. It is a low plant with ascending laterals and broad leaves. The habit is well represented in the photograph reproduced in plate 6. The leaves are broad proportional to their length, rounded and abruptly narrowed into a very short point above, but gradually tapering to a broad clasping base below with rounded but not prominent basal lobes (hardly to be termed auricles). A comparison of the habit-photograph mentioned above with the plate of Comes will show how U. C. B. G. $\frac{22}{07}$ differs from his var. *macrophylla.* The flowers are deep rose color to red with stout tube and abruptly swollen, broad infundibulum, and with the limb almost pentagonal. It is marked at the very shallow sinuses with triangular depressed whitish areas as represented in the figure of Comes (1899, pl. I and pl. VIII). The capsule is broad, short, rounded and nearly enclosed in the calyx (cf. Comes, 1899, pl. I). This will be referred to as *Nicotiana Tabacum* var. *macrophylla.*

Nicotiana angustifolia

U. C. B. G. $\frac{68}{07}$.—The seed whence the plants designated by this number have sprung was obtained in 1907 from the authorities of the Jardin Botanique de la Faculté de Medecine du Lyon, under the name of *Nicotiana angustifolia.* It is the plant known early under the name of *Petum angustifolium* (cf. Clusius, 1605, p. 310) and has passed by the name under which we we received it since 1768 (cf. Miller Dict. ed. viii). It belongs to the group of *Tabacum* varieties placed under *N. fruticosa* or those with distinct and non- or only slightly alate petioles. It is very near the *N. Tabacum* var. *fruticosa* of Comes (1899, p. 8, pl. I, III), but that is evidently not the *Nicotiana Tabacum* var. *fruticosa* of Hooker (1876, pl. 6207) which is a plant with a sessile clasping leaf and much nearer to U. C. B. G. $\frac{71}{05}$. It is not the same plant as the one in the Linnaean Herbarium preserved as the type-specimen of *N. fruticosa* L. It is impossible for me, at present, to attempt to unravel the synonymy of this plant further, but it will be quoted in the following pages as *N. angustifolia,* using this binomial simply as a convenient

designation for the present. It is not *N. angustifolia* Ruiz & Pavon, however.

N. angustifolia, or U. C. B. G. $\frac{68}{07}$, is a comparatively low plant, about three feet in height as a rule, of decidedly corymbose habit, i.e., the main axis is of limited growth in height and is soon equalled or even overtopped by several (or all) of the laterals. A young plant is represented in the photograph reproduced in plate 7. The leaves are distinctly petioled and the petiole is naked, at least in the lower half or third. The blade of the leaf is obliquely ovate-lanceolate, tapering gradually into a long, laterally curved point. The base of the blade is broadly rounded and is decurrent along the upper half (or even two-thirds) of the petiole as a narrow wing. The blade is more or less conduplicate. The upper leaves are shorter-petioled, narrower, and shorter-pointed than the lower, while the uppermost are often reduced to very narrow linear shapes. The petiole is provided with two sharp angles at the junction of the upper (almost flat) and the lower (very convex) surfaces. There are no auricles at the base of the petiole. The panicle is crowded with slender flowers. The calyx is narrow and with long slender lobes. The tube of the corolla is slender below, expanding gradually and not considerably, into a narrow infundibulum. The tube of the corolla in *N. angustifolia* is the most slender, especially as to the *infundibulum,* of any of the *N. Tabacum* group. The limb is very light pink and deeply divided into narrow lobes which are broader below but above are abruptly narrowed into long, slender lanceolate tips.

In habit U. C. B. G. $\frac{68}{07}$, or *N. angustifolia* as we may call it, is near to U. C. B. G. $\frac{22}{07}$, being more slender, but in its petioled leaves, its crowded panicle, its slender flowers with narrowly and deeply lobed limb, it is most distinct from all others of the *Tabacum*-group under cultivation at present in the U. C. B. G.

Nicotiana Tabacum var. **macrophylla purpurea**

U. C. B. G. $\frac{25}{06}$ was received from the Missouri Botanical Garden in 1906 under the name of *Nicotiana sanguinea.* It is

one of the plants usually known in gardens under that name. It is tall, six feet high or over, with large deep-red flowers, of the same shape as, though with rather deeper color than, those of *N. Tabacum* var. *macrophylla* (cf. U. C. B. G. $\frac{22}{07}$). *N. sanguinea* is designated by Comes (1899, p. 20) as "*N. Tabacum* var. *macrophylla purpurea,*" but it is to be noted that he expressly states that his *N. Tabacum* var. *macrophylla purpurea* includes both *N. sanguinea* and *N. purpurea* of the gardens, but only partially as to each. These two garden tobaccos vary in height, robustness, and color of flower. Even the shape of the flower varies among the different plants referred here. The leaves are ample, with fairly long, broad-winged petiole, broadly ovate blade, which is more or less cucullate at the tip. There are combined in this plant characters of our *N. angustifolia* (U. C. B. G. $\frac{68}{07}$) as to petiole, *N. Tabacum* var. *brasiliensis* (our *Brazilian*, U. C. B. G. $\frac{71}{05}$) as to cucullate tip, tallness, and perhaps also the wing on the petiole, and *N. Tabacum* var. *macrophylla* (cf. U. C. B. G. $\frac{22}{07}$) as to flowers. I have produced plants similar to this, but lacking tallness and the cucullate tip to the blade of the leaf, in F_2 from crosses between U. C. B. G. $\frac{68}{07}$ and $\frac{22}{07}$. *N. sanguinea,* at least so far as U. C. B. G. $\frac{25}{06}$ is concerned, is a poor and uncertain seeder. This leads one to suspect a possible hybrid origin. It has bred true in the U. C. B. G., however, for several years. This tobacco is grown, chiefly at any rate, as an ornamental plant. U. C. B. G. $\frac{25}{06}$ is well represented in plate 8. The two garden species, known as *N. sanguinea* and *N. purpurea* vary in height and robustness but those with the darker flowers are called *N. purpurea* while those with the lighter flowers are called *N. sanguinea.*

Section II. Rustica G. Don

In this second section of the genus are placed all the yellow-flowered species and varieties. The color is usually simply yellow, but, at times, in certain species, it may be mixed with red or even with white. The shape of the corolla varies much. It may be infundibuliform, hypocraterimorphous, ventricose, or

even nearly tubular. The corolla of all species of *Nicotiana* is more or less irregular, being slightly zygomorphous merely in most cases, but in two species of the *Rustica*-section, viz., *N. glutinosa* and *N. tomentosa,* it is decidedly irregular as well as being deeply tinged with red. The flowers are in simple or panicled racemes. Of about sixteen species credited by Comes (1899) to this section, six are commonly cultivated in gardens and are represented in the collections of the U. C. B. G., together with several fairly distinct varieties.

Nicotiana rustica L.

Many varieties and forms of *Nicotiana rustica* are cultivated and while, perhaps, the variability of the plants included under this name is not quite so great as is the case with those included under *N. Tabacum,* yet it is certainly very great. Forms of this species were cultivated and used for smoking by the North American Indians from Mexico and Texas north along the western banks of the Mississippi River to Minnesota, eastward to the Atlantic seaboard and north thence to Canada. It was the first tobacco cultivated by the English in Virginia, although it was soon supplanted by varieties of *Nicotiana Tabacum* brought from northern South America and the West Indies. Its culture spread to Europe, where it is cherished locally as a peasant tobacco, as well as to Asia and to Africa. It is to be expected that many varieties may be found in a species so widely and so long cultivated. Comes (1899, pp. 20-24) has distinguished six varieties, all of which have been grown in the U. C. B. G. from seed kindly furnished by Professor Comes himself. Many sowings of this species of seed from other sources have also been made and the following seven varieties have been selected for further work.

Nicotiana rustica var. **asiatica**

U. C. B. G. $\frac{12}{07}$.—*Nicotiana rustica* var. *asiatica* Schrank (1807, p. 264), as interpreted by Comes (1899, p. 22, pl. II, XII), is fairly tall, with ample leaves, which are more or less heart-shaped, and a spreading panicle. The seed came from Pro-

fessor Comes and the plant has held its characters in the U. C. B. G. since 1907. It comes near to *N. rustica* var. *jamaicensis* Comes (U. C. B. G. $\frac{15}{07}$) in its spreading habit, but the leaves are more cordate than in the latter variety. The photograph reproduced in plate 9 represents one of the less ample plants.

Nicotiana rustica var. brasilia

U. C. B. G. $\frac{13}{07}$.—*Nicotiana rustica* var. *brasilia* Schrank (1807, p. 264) as interpreted by Comes (1899, p. 22, pl. II, XI), is a fairly tall, robust plant, very distinct from the other varieties of *N. rustica*. The stem is stout, clothed below with large, thick heart-shaped leaves which are decidedly rugose. The panicle, when well developed, is long tapering and of massive appearance, with crowded flowers. This is well shown in the photograph reproduced in plate 10. Later, by the growth and flowering of the laterals, the panicle appears more spreading, as represented in the plate of Comes (1899, pl. XI). The seed came from Professor Comes and the plants have preserved their characteristics when bred in the pure line, in the U. C. B. G., since 1907.

Nicotiana rustica var. humilis

U. C. B. G. $\frac{14}{07}$.—*Nicotiana rustica* var. *humilis* Schrank (1807, p. 264), as interpreted by Comes (1899, p. 23, pl. II, XIII), is a fairly robust plant, but of low stature and early blossoming and ripening. A fairly typical plant is represented in plate 11. The leaf is broad and ovate, being broadly, but slightly cuneate at the base. The panicle is comparatively simple. This variety is nearer to *N. rustica* var. *jamaicensis* Comes than to any other variety, but is more simple in habit and with leaves more perfectly ovate with the base more cuneate and even. The seeds were obtained from Professor Comes and the plant has held its characteristics in the U. C. B. G. since 1907. This species, at least as represented by No. $\frac{14}{07}$, comes near to *N. rustica* var. *texana*, at least as represented by U. C. B. G. $\frac{17}{07}$. The two plants are still being studied.

Nicotiana rustica var. jamaicensis

U. C. B. G. $\frac{15}{07}$.—*Nicotiana rustica* var. *jamaicensis* Comes (1899, p. 21, pl. II, X) is a plant not always to be readily distinguished from *N. rustica* var. *asiatica*, on the one hand, and *N. rustica* var. *humilis* on the other. It has a less spreading habit than the former, but more than the latter. Its leaves are not so cordate as those of the former, but at the same time they are more rounded than those of the latter. It holds its characters when bred in the pure line as it has been in the U. C. B. G. since 1907. The seed was received from Professor Comes himself. It is shown in plate 12.

Nicotiana rustica var. scabra

U. C. B. G. $\frac{16}{07}$.—*Nicotiana rustica* var. *scabra* (Cav.) Comes as interpreted by Comes (1899, p. 23, pl. II, XIV) is a most characteristic plant and seems worthy of independent specific rank, so much does it differ from the other varieties of *N. rustica*. It is a tall plant and decidedly pruinose. While the plate of Comes (*loc. cit.*) represents a plant of rather spreading habit, the plants of the U. C. B. G., grown from seed sent from Portici by Professor Comes himself, are more strict; some of them are in fact of very narrow habit. Unfortunately I have no photograph to represent this number as yet, but a fairly typical specimen of this variety is represented by U. C. B. G. $\frac{26}{06}$ and is reproduced in plate 13. The var. *scabra* is not only distinct in its general habit, size, and pruinose appearance, but it has a bluish purple color to the buds and young twigs and smaller and more crowded flowers, which are greenish yellow. It lacks glands except on the flowering axes, being clothed elsewhere by a thick and compact covering of white, slender hairs abruptly bent at the middle. Above, among the flowers, these are mixed with the ordinary stalked, multicellular glands commonly found in the species of *Nicotiana*.

Nicotiana rustica var. **texana**

U. C. B. G. $\frac{17}{07}$.—*Nicotiana rustica* var. *texana* (Naud.) Comes, as interpreted by Comes, is shown in plate 14, representing a plant from pedigreed seed kindly sent by Professor Comes in 1907. It is a coarse plant, next lowest in stature to var. *humilis*, to which it approaches more nearly than it does to other varieties of *N. rustica*. Its habit is looser, as to the panicle, and the leaves are more rounded at the base. The flowers are more slender than those of *N. humilis*. On the whole, however, the two varieties are very close to one another. (cf. plate 14).

Considering all the varieties and forms of *Nicotiana rustica* which I have been able to obtain and cause to grow in the U. C. B. G., the varieties *brasilia* and *scabra* are the most distinct, yet all have more or less definite points of distinction. Most of the plants of this species from other sources which have been grown may be referred more or less definitely to one or other of the six varieties enumerated above or seem to be intermediate between some two of them. One other stock, besides those mentioned above has been retained, viz., *U. C. B. G.* $\frac{169}{08}$.

Nicotiana rustica var. **pumila ?**

U. C. B. G. $\frac{169}{08}$.—This is referred with doubt to *Nicotiana rustica* var. *pumila* Schrank (1807, p. 264) and is represented in the photographs reproduced in plates 15 and 16. This plant is the lowest of all the members of the *N. rustica* assemblage which have come under my observation. It is 12 to 14 inches high, matures early, and is loose in habit. Its leaves are ovate-lanceolate and unequal at the base. They are small compared with those of the other varieties of *N. rustica*. For three seasons, bred in the pure line, it has retained its lowly habit, earliest flowering of all the varieties of *N. rustica*, and its narrow leaves.

Nicotiana Langsdorffii Weinm.

This species was described by Weinmann (p. 323) and by Schrank (pl. 72) in 1819 as coming from Brazil and was intro-

duced into cultivation the same year apparently. It was originally collected by Langsdorff, who sent the seeds to Weinmann. The description speaks of the flowers as green and the anthers as azure. The type of the species is a fairly well-known garden plant. It has been cultivated in the U. C. B. G. under various numbers, such as $\frac{22}{02}$ and $\frac{102}{05}$. The latter number is well represented in the photograph reproduced as plate 17.

N. Langsdorffii is a plant of three or four feet in height, of loose and spreading habit. Its leaves are elliptical-lanceolate, patent, narrowed and sessile by a long decurrent base. They are decidedly rugose above. The corollas are funnel-shaped below with a gibbous ring above and a concave, spreading limb slightly notched in five broad, shallow lobes. They are greenish yellow and pendent, or at least nodding. The pollen is azure. The capsules are for the most part 2-celled, but 3-celled capsules are not uncommon.

While the type is unmistakable and is well represented in the plates in the *Botanical Magazine* (cf. Sims, 1821, pl. 2221 and 1825, pl. 2555), there are plants often referred to it which Comes has mentioned as varieties.

N. Langsdorffii var. *grandiflora* Comes (1899, p. 28) is the plant of the gardens usually known as *N. commutata* Fischer et Meyer (1846, III, p. 377). It is a plant of less slender and less spreading habit, larger flowers, which are more deeply notched, more decidedly zygomorphous, and with the limb more spreading. The outside of the corolla is greenish yellow as in the type, but the inner (upper) surface of the corolla is milk-white. The flowers also are ascending, not pendent or hanging. The pollen is slightly bluish, not at all azure, but the anther coats are purplish brown. Altogether, the characters recall those of *Nicotiana alata* var. *grandiflora* Comes (1899, p. 37) which is *N. affinis* Moore (1881, p. 141, fig. 31), except that the flowers are smaller and more decidedly yellow outside. It may be of hybrid origin. It is said to have been known in gardens since 1835, but its native country is uncertain. In the U. C. B. G., it is represented by number $\frac{107}{08}$ (cf. plate 18), where its behavior is being studied and about which it is hoped to publish

something at a later date. Thus far it has produced both whites and pure yellows. Lock (1909) has made some experiments in crossing *N. Langsdorffii* and *N. alata,* with very interesting results as to corolla-shape and color, and also as to color of the pollen. Both the F_1 and the F_2 generations in Lock's experiments presented intermediates.

N. Langsdorffii var. *longiflora* Comes is another intermediate sort of variety described by Comes (*loc. cit.*). I have not had any plants which answer exactly to his description, but under No. $\frac{173}{08}$ U. C. B. G. (cf. plate 19), there appeared yellow-flowered forms (even the inner, or upper, surface of the limb being yellow) which comes close to it, as do also certain plants cultivated under the number $\frac{70}{06}$ U. C. B. G., which also have given both yellow and white-limbed flowers. All these are being bred in pure line to be reported on later.

Nicotiana paniculata L.

This well-known and widely cultivated species has been grown in the U. C. B. G. under several different numbers and from several different sources. No. $\frac{106}{05}$ U. C. B. G. is well represented in the photograph reproduced in plate 20. It is a spreading plant up to three or four feet high, the panicle being very effuse. The leaves are broad and slightly cordate, moderately long petioled. The flowers are pale yellow and long tubular, being slightly gibbous just below the limb. The limb is narrow, at first concave, but flattened or somewhat reflexed in full anthesis, broadly and very slightly rounded five-lobed. The capsule is narrow.

This species is reported to have been under cultivation since the middle of the eighteenth century, having been discovered in Peru in 1752. The plant in the Linnaean Herbarium is exactly the one grown in the U. C. B. G. It is said to be used as a tobacco for the pipe, being mild and of exquisite aroma. It remains constant when cultivated, although at times the flowers are curved nearly into a circle.

Nicotiana glauca Graham

N. glauca is a tree tobacco, since it is a perennial and forms a trunk of considerable height and girth. It has spread from its original habitat into a considerable number of tropical and warmer temperate countries. It is probably a native of central South America. It is a common escape in Southern California where it is thoroughly naturalized and commonly reaches a height of ten or twelve feet. It grows fairly well in central California too and has appeared in abundance in San Francisco in the section burned over in 1906. The stem is woody and much branched. The leaves are long petioled, ovate-lanceolate, glabrous and glaucous. It is the most nearly glabrous *Nicotiana* we have cultivated in the U. C. B. G. (No. $\frac{5}{10}$). The flowers are pale yellow, long tubular, slightly gibbous above and with the almost pentagonal limb deeply concave. In flower, it comes nearest to *N. paniculata.* W. J. Hooker has accurately figured and described it (1827, pl. 2837). Comes (1899, p. 27) has described three varieties which I have not, as yet, been able to distinguish.

Nicotiana glutinosa L.

This is one of the most peculiar of the annuals of the section *Rustica* in its foliage and its flowers. It is a very robust plant, as represented in the photograph reproduced in plate 21. The leaves are broadly and deeply cordate and abruptly acuminate. The whole plant is pubescent-villose and extremely glandular sticky. The racemes are long, circinate at the tip, and with the flowers alternate in two ranks on the same side. The flowers are unlike those of any *Nicotiana* in shape except those of *N. tomentosa.* They are short cylindrical below, suddenly swollen above, where they open out in an irregular obliquely one-sided funnel. The limb is fairly bilabiate, the stigma and anthers being connivent just under the middle lobe of the upper lip. The color is light yellow tinged with deep red. The flowers easily fall especially when there is a drop in temperature. Under No. $\frac{79}{07}$ it has been cultivated in the U. C. B. G., in the

pure line, for several years and retains its characters perfectly. The plant in the Linnaean Herbarium is exactly the one cultivated in the U. C. B. G. and elsewhere, and which passes universally under this name.

Nicotiana tomentosa Ruiz & Pavon.

N. tomentosa is a second "tree tobacco" rivalling *N. glauca* in height and exceeding it in display as a foliage plant because of its huge leaves. U. C. B. G. $\frac{1903}{08}$ is the number applied to plants of this species grown in Berkeley, where one plant has survived three winters outside, the first under protection of a cheese-cloth tent and with some heat at nights, the second and third without protection. It is now a bushy plant of spreading habit, about twelve feet high, and has blossomed thrice, but since it begins to blossom in midwinter, few of the earlier blossoms arrive at anthesis. Some of the latest do, however, and in the present year (1912), it has produced abundant panicles for several months. The shape of the corolla is nearest to those of *N. glutinosa,* being obliquely inclined, very gibbously inflated into a broad funnel above and nearly bilabiate. The color is light yellow tinged with red. The style and stamens are exserted, projecting fully as much as the length of the corolla. Both flowers and leaves are well figured by Hooker (1892, pl. 7252). On account of the peculiarities of the flower, Sprengel (1817, p. 458) made it the type of his new genus, *Lehmannia.* It was named by André (1888, p. 511) *Nicotiana colossea,* and it has appeared in gardens and has been cultivated as a foliage plant under this name. It is usually raised under glass and placed outside only in the warmer season. It begins to flower in the U. C. B. G. in December and continues to do so for several months. It is a native of Brazil and Peru. A small plant grown from a cutting is represented in plate 22.

Section III. Petunioides G. Don.

The species of Nicotiana belonging to the *Petunioides*-section have salver-shaped corollas, which are white or tinged with red or purple, arranged in racemes or panicles. In this section,

I have included the section *Polidiclia* of G. Don, an arrangement which seems to me natural and which I shall hope to justify further on (cf. also Miers, 1846, p. 182 and East, 1912). Of the twenty-four species included by Comes (1899) in these two sections, ten are cultivated in the U. C. B. G.

Nicotiana noctiflora Hook?

The description of this species as given by W. J. Hooker (1827, pl. 2785) is such that I hesitate to apply the name to the plants cultivated for several years in the U. C. B. G. under No. $\frac{9}{07}$. The principal differences are in the corolla lobes and in the inflorescence. The corolla lobes are represented as broad and emarginate by Hooker. In our plant they are broad and bluntly pointed, but the blunt point is revolute and the superficial appearance is of a blunt and emarginate lobe. The inflorescence represented in Hooker's plate is more paniculate than I find in the U. C. B. G. plants. The leaves appear to be very much the same in both.

U. C. B. G. $\frac{9}{07}$ came from seed sent by Professor O. Comes and was labelled "*Nicotiana noctiflora* var. *albiflora.*" Its habit is low (about two feet in height), rather effuse, and sprangly. The leaves are coarse, especially the lower ones. They are elliptical-lanceolate to simply broadly lanceolate, sessile and slightly clasping at the base, more or less bullate above, slightly toothed, sinuous and undulate, with sparse, coarse prickly hairs. The upper leaves are narrowly linear-lanceolate, very much and coarsely crisped. The flowers are in long simple racemes. The corolla is salver-shaped, with a slender tube, about double the length of the calyx, and expanded gently at the summit. The five lobes of the limb are broad and deep, abruptly contracted at the tip, which is revolute, thus giving the lobes a certain appearance of being broad and obcordate. The corolla is reddish purple without and white, or slightly purplish, within. U. C. B. G. $\frac{9}{07}$ is a near relative of N. *longiflora,* from which it is to be distinguished particularly by its strictly annual character, lower habit, its lack of a long persistent basal rosette of radical leaves, and much shorter corolla. Although it seems to pass for *N.*

noctiflora, it may well be doubted whether it is identical with the plant described under this name by W. J. Hooker (*loc. cit.*). So far as flower and inflorescence is concerned, Hooker's plant seems to be nearer to *N. acuminata.* Hooker's plant is credited as being perennial, ours is annual. The flowers open at about 7:30 P.M. and close by 8 A.M They are odorless. U. C. B. G. $\frac{9}{07}$ is shown in plate 23.

Nicotiana longiflora Cav.

The present species is fairly well known in botanical gardens and as a weed in warmer countries. It has appeared in the eastern United States as a ballast weed. It has been grown in the U. C. B. G. under several numbers, from as many different sources. No. $\frac{100}{05}$ has been the principal cultivation and the plant (in daytime with its flowers closed) is well represented in the photograph reproduced in plate 24. One characteristic of *N. longiflora* is very striking in contrast with other species of *Nicotiana* cultivated in the U. C. B. G. and that is, the forming of a compact rosette of large, coarse leaves which lie flat on the ground and persist for a considerable time before the flowering stems arise from it. The rosette persists for most of the first year and is well represented in the figure just quoted. The flowering stems are spreading, bearing narrower leaves than the radical ones, and the loose panicle bears somewhat distant flowers with long, slender, bluish-purple corollas.

The radical leaves are broadly lanceolate or oblanceolate, coarsely bullate and rugose above, undulate, smooth but with coarse spine-like glandular hairs on surface and margins. The tube of the corolla is four to six times as long as the calyx and proportionally slender. The broad spreading limb is deeply divided into five moderately broad blunt-pointed lobes, which are somewhat recurved. The flowers open only at night. Occasionally a 3-celled capsule is found. In the U. C. B. G., No. $\frac{100}{05}$ has persisted as long as three years, but no more. It usually lives for two years, at least.

U. C. B. G. $\frac{104}{08}$ seems to be the *N. longiflora* var. *acutiflora*

of Comes (1899, p. 44). It was received under the name of *Nicotiana acutiflora,* as have been also one or two other plants from other sources. Our plant is certainly very near to *N. longiflora,* as cultivated in the U. C. B. G., differing chiefly in the decidedly more yellowish green stems and foliage, flowers greenish white with only a slight tinge of purple in some of them, more sinuous lobes recurved to the limb of the corolla, and more conduplicate and twisted cauline leaves. Its perennial character in the U. C. B. G. is limited to two or three years, as in typical *N. longiflora.*

Nicotiana alata Link et Utto.

Nicotiana alata in one form or another has been a favorite in cultivation for a long period, partly for ornamental purposes, but partly, it is claimed by many authorities, to provide the Persian tobacco so highly esteemed for its delicacy and perfume. The most commonly cultivated variety is the plant called *Nicotiana affinis* Moore (1881, p. 141, fig. 31). Comes (1899, p. 37) has designated this as var. *grandiflora* of *N. alata.* Another variety of *N. alata,* according to Comes, is *N. persica* Lindley (1833, pl. 1592). This is *N. alata* var. *persica* (Lindl.) Comes (1899, p. 36). The differences between these three (?) sets of plants seem to be largely in the more or less amplexicaul base of the leaf as well as its varying degree of decurrence, the varying size of the flower, and the variety exhibited in the disproportionality (zygomorphism) between the upper three lobes of the limb of the corolla and the lower two. In the type of the species, all five lobes are said to be very nearly equal, obtuse and not emarginate and the limb very little oblique; in var. *persica* the limb is said to be decidedly oblique, the lobes scarcely unequal but strongly emarginate; in var. *grandiflora* the tube of the corolla is said to be longer and stouter, the limb very oblique, broader, with larger lobes, the lower two being much the larger, but only slightly emarginate.

I have cultivated several different sets of *N. alata* in the U. C. B. G. and have obtained a variety of plants, but without being able to separate the varieties satisfactorily. The plants, clearly and distinctly belonging to *N. alata,* are all of var.

grandiflora or very close to it. What seems in many ways to be the var. *persica* is represented by U. C. B. G. $\frac{107}{08}$. It was received under the name of *N. viscosa*. I have referred to it in the present account under *N. Langsdorffii* var. *grandiflora*. The corolla tube is provided with a gibbous ring at the summit. It is to be suspected as of hybrid origin, the most probable parents being *N. alata* var. *grandiflora* and *N. Langsdorffi*. It has given plants both yellow and milk-white for the color of the upper surface of the limb of the corolla during cultivation in the U. C. B. G.

N. alata var. *grandiflora* is represented by U. C. B. G. $\frac{98}{05}$ (cf. photograph, reproduced in plate 25), and by U. C. B. G. $\frac{1}{06}$. They are both clearly the *N. affinis* of the gardens, but differ slightly from one another. The flowers are large, with the tube gradually enlarging up to the limb and with very little trace of a gibbous swelling at the very top and that only on the upper side. The tube is greenish yellow without and the lower (outer) surface slightly purplish. *N. alata* var. *grandiflora* has been crossed with *N. Forgetiana* Hemsley (1905, pl. 8006) to produce the brilliant red plant of the gardens known as *N. Sanderae*. This hybrid has been grown in the U. C. B. G. under several numbers and has exhibited a considerable variety of form and color of flower and some variability in habit and fertility. All the true *N. Sanderae* show the influence of the red-flowered parent (*N. Forgetiana*) not only in color, but also in the strongly developed gibbous ring in the throat of the corolla just below the limb. Other hybrids of *N. alata* var. *grandiflora* are known in gardens, with flowers varying from white, through pink and red to dark purple (bluish in fading). In size and shape, the flowers, as well as the habit, vary very little from typical *N. alata* var. *grandiflora*. U. C. B. G. $\frac{174}{08}$ is such a hybrid, giving uniformly dark-red flowered plants in pure-line breeding.

Nicotiana acuminata (Graham) Hook.

Our knowledge of *Nicotiana acuminata* is based on W. J. Hooker's (1829, pl. 2919) description and plate. In the U. C.

B. G., there have been cultivated several plants which seem certainly to belong to the same species. They differ from one another slightly, but chiefly in the varying diameter of the limb of the corolla. Comes (1899, p. 39) has described two varieties based on this character and we have followed him in adopting designations for our plants. The *acuminata*-group is to be distinguished from the *longiflora*-group by having the lobes of the limb of the salver-shaped corolla comparatively shallow and rounded. The varieties of *N. acuminata* have petioled leaves, whose blades are almost or quite cordate below but ovate-lanceolate to narrowly lanceolate above. The plants are rather spreading at maturity, as represented in plate 26. The seed of the type of the species (apparently var. *parviflora* Comes) came originally from Chili (Hooker, *loc. cit.*). One of the varieties (var. *grandiflora* Comes, cf. plate 26) probably originated (or segregated) under cultivation, but one or more varieties occur wild in California. Since the subject of *N. acuminata* and its varieties is to be treated by T. H. Goodspeed in a paper about to appear in this series, nothing further will be said about its characters here. Plants belonging to this species appear in botanical gardens under the names of *N. suaveolens* and of *N. vincaeflora*, Australian plants which were at one time cultivated but of which I have been unable to get reliable seed. I suspect that most of the plants cultivated under these names belong to *N. acuminata*, from which they are to be distinguished by their lack of a petiole.

Nicotiana attenuata Torr.

This is a widespread species of Western North America, extending from New Mexico, Arizona, and southern California, east as far as Colorado, and north through Wyoming to southern British Columbia. It grows in arid and desert localities, as a slender, often decidedly bushy herb of straggling habit. It is well represented by Watson (1871, pl. 26, fig. 1, 2) in his figure in the *Botany of King's Expedition*, except that the flowers seem to be sharp-lobed and funnel-shaped, while the large very extremely swollen-based glandular hairs are not

represented. The large swollen based hairs are most characteristic and constitute a mark of identification. They are usually very conspicuous upon the calyx. The plant bears a certain fairly close but superficial resemblance to the last (*N. acuminata*), especially to the smallest flowered varieties. It differs from it in the swollen-based glands just mentioned, in the lower leaves never being so broadly ovate or even cordate at the base, and in the shorter, stouter tube of the corolla. The leaves are petioled and lanceolate or ovate-lanceolate and the lobes of the limb of the corolla are broad and shallow, but not emarginate. It has never been cultivated to any extent and does not grow well in the adobe soil of the U. C. B. G. Two numbers, viz., U. C. B. G. $\frac{78}{09}$ and $\frac{46}{11}$, have been grown with fair success. Both numbers are from plants used by Indians for smoking, the former from seed from Oregon, the latter from seed from British Columbia. It seems to have been smoked by the Indians throughout its range to some extent, at least wherever it was the chief or only species of *Nicotiana* to be obtained. In the northern part of its present range it was undoubtedly introduced by the Indians.

Nicotiana Bigelovii (Torr.) Watson.

One of the most interesting of all the *Nicotiana* species cultivated in the U. C. B. G. is this species of California and, to some extent perhaps, of adjacent states. It has been cultivated in various forms and under various numbers in the U. C. B. G. since 1905, these latter years in pure lines. I do not think that it has been successfully cultivated elsewhere to any extent (cf. however Comes, 1899, p. 43 and East, 1912) and the only time I saw the name was in the seed-list of a botanical garden. I obtained some of the seed but the plants proved to be *N. longiflora*. It does not grow readily or uniformly in the U. C. B. G., but it has always given some results and these have been of such interest that I expect to give them more in detail later.

What passes for the type of *Nicotiana Bigelovii* is a large and tall, coarse plant with large white or purplish (outside) corollas which are five-lobed. The leaves are sessile and usually

tapering towards the base, although, in some plants, some of the leaves are truncate at the base and partly clasping. The capsule is large and two- or three-celled. It generally grows in sandy banks or bottoms of rivers, overflowed in spring, but dry in summer. There are several variations of the type upon which it is hoped to report later. U. C. B. G. $\frac{40}{05}$ represents such a plant, while U. C. B. G. $\frac{60}{07}$ a, represents a lower, spreading plant which may possibly be nearer the strict taxonomic type. The figures given by Watson (1871, pl. 26, fig. 3, 4) in the Report of the Botany of King's Expedition resemble U. C. B. G. $\frac{60}{07}$ a, more nearly than any other and the type (or cotype) in the Gray Herbarium of Harvard University seems to be the same. These plants are the more common in central California. *N. Bigelovii* var. *Wallacei* Gray is a slender plant, with slender, narrower corolla, with elongated deltoid leaves. This is the more abundant form in Southern California. U. C. B. G. $\frac{43}{05}$ represents this type.

Nicotiana quadrivalvis Pursh.

Nicotiana quadrivalvis seems to be a lost species in nature. It was described by Pursh (1814, pp. 141, 142) from the plants collected by Lewis and Clark in their expedition across the continent. Lewis and Clark got their plants from the Ricaree Indians who cultivated it. The type-specimen is still preserved in the Herbarium of the Academy of Natural Sciences at Philadelphia, where I have had the opportunity of examining it through the courtesy of Mr. Stewardson Brown. It seems exactly like the plants developed in the U. C. B. G. and somewhat unlike the plants grown in the past in various botanical gardens. It was introduced into gardens in 1811 (cf. Don, 1838, p. 466) and was figured by Sims (1816, p. 1778). Lehmann (1818, p. 45, pl. 4) also described and figured it. How long it persisted in botanical gardens is not certain, but it seems to have been extensively cultivated, judging from herbarium specimens. It is still offered in some seed-lists, but seed obtained from such lists have given me only varieties of *N. Tabacum* or *N. rustica.*

Comes (1899, p. 54) indicates that he has seen it in the living condition. Seed received from him failed to germinate in the U. C. B. G., even after repeated trials. East (1912, p. 244) has just stated that he succeeded in producing plants from Italian seed and that these were so close as to seem of the same species with a derivative of *N. Bigelovii* produced in the U. C. B. G., of which East was furnished with seed.

As to the wild plant, I have considerable doubt as to its ever having existed. In various floras it is listed without special comment, but very few specimens other than those from botanical gardens are to be found in the herbaria and even such other specimens are likely to have been from Indian cultivation. Some of such specimens, however, are either robust *N. Bigelovii* or *N. multivalvis.* Gray (1876, p. 546) suggests that it is merely a cultivated variety of *N. Bigelovii* to which it is very close in every character except that of the four-celled capsule and its tendency to have more than five lobes to the corolla. My experience with *N. Bigelovii,* especially U. C. B. G. $\frac{35}{05}$, seems thoroughly to support this statement, since *N. quadrivalvis* has appeared in a pedigree of *N. Bigelovii* (cf. also East, 1912, p. 245 et seq.).

Nicotiana multivalvis Lindl.

This plant is more commonly assigned as a variety under *N. quadrivalvis,* which it resembles closely except in the many-celled indehiscent capsule, in which the cells are arranged in both an inner group and an outer row and the many-lobed limb of the corolla. In fact, *N. multivalvis* (cf. Lindley, 1827, pl. 1057) seems like a monstrous form of *N. Bigelovii.* Yet it is reproduced uniformly from the seed. U. C. B. G. $\frac{90}{06}$ and $\frac{143}{07}$ have been grown for several years in the U. C. B. G., mostly in the pure line, giving constant results. Gray (1876, p. 546) suggests that "*N. Bigelovii* is perhaps the original of it," and I feel that he is right, since it has appeared in the pedigreed cultivation of *N. Bigelovii* in the U. C. B. G.

N. multivalvis has been cultivated in botanical gardens since 1826 (cf. Don, 1838, p. 467) and still persists. The first seed were procured by David Douglas (1836, p. 92), who obtained

it in 1825 on the banks of a small branch of the "Multnomak River," one of the southern tributaries of the Columbia River. The plants cultivated today are, with little doubt, descended from the plants grown from the seed collected by Douglas. No plants of *N. multivalvis* are found wild at the present time, and it is more than probable that even in the time of Douglas it was not known except in Indian cultivation. It is still cultivated and used ceremonially by certain Indian tribes.

Nicotiana repanda Willd.

Lehmann (1818, p. 40, pl. III) is responsible for the publication of this species which Willdenow apparently christened as an herbarium specimen. The native country of the type specimen is given as Cuba. The species grows in Mexico and southwestern Texas, where seeds were obtained through the kindness of Professor F. D. Heald of the University of Texas. The plants are not easily grown but have been continued on under U. C. B. G. $\frac{79}{09}$. They agree with Lehmann's figure (*loc. cit.*) as well as with that of Sims (1823, pl. 2484). They are well represented by the photograph reproduced in plate 27. Our plant is probably the *N. repanda* var. *pandurata* (Dunal.) Comes (1899, p. 47).

Nicotiana trigonophylla Dunal.

In the southwestern United States and northern Mexico, in the drier regions, there grows a species which varies quite a little, which has been, and even still is, used by certain Indian tribes for smoking. This is *Nicotiana trigonophylla.* It has been grown, but with difficulty, in the U. C. B. G. for several seasons under Nos. $\frac{95}{07}$ and $\frac{6}{09}$. The seed of the former was from San Bernardino County and of the latter from Inyo County, both of the State of California. In both cases it was from wild plants.

In 1911 by planting in soil well underdrained and by using cheese-cloth protection, the plants were grown successfully and some of them with protection have even withstood the winter.

The species is correctly placed in the *Petunioides*-section of

Nicotiana, although the flowers are a yellowish white. The individuals vary in being more green or more glaucescent. They are straggly, with narrow, broadly lanceolate leaves narrowed at the base and then suddenly expanded into broad, partly clasping auricles. The flowers are in more or less incurved, one-sided racemes. The corolla tube is almost straight tubular, with the limb spreading at right angles or slightly deflexed in full anthesis. The lobes of the limb of the corolla are broad, obtuse, and shallow. The flowers are deep creamy yellowish white. The capsule is nearly or entirely enclosed in the calyx, varying in this respect. Var. *pulla* Comes (1899, p. 49), var. *sordida* Comes (*loc. cit.*) and var. *ipomopsiflora* Comes of *N. trigonophylla* and *N. Palmeri* Gray (1886, p. 242) seem to belong to the same species.

Nicotiana sylvestris Speg. & Comes.

This is one of the most important of the showy species of *Nicotiana* for garden culture. Spegazzini sent the seeds from Argentina to Comes in 1897 (cf. Comes, 1899, p. 35), and the plant was soon widely distributed in botanical and other gardens. It has been grown continuously in the U. C. B. G. since 1901. It is a tall, shortlived perennial with long, slender white flowers, pleasantly fragrant after dark. A slender plant just coming into flower is represented in the photograph reproduced in plate 28. The figure of Comes (*loc. cit.,* p. 34) does not well represent the proportions of height, length of leaf, and length of flower as found in the plants cultivated in the U. C. B. G. The plate of J. D. Hooker (1899, pl. 7652) is better in these respects, but the habit-figure does not seem characteristic. The plant represented from the U. C. B. G. is young; older plants branch and become more bushy. The flowers are long and white, with a slight tinge of yellowish outside. The tube is slender, enlarging slightly and gradually above the middle but gradually contracting below the top. The limb is moderately broadly lobed one-third to one-half way in from the margin. The lobes are broadly triangular. The flowers are pendent in bud,

ascending to nearly horizontal as anthesis proceeds. While they are open in the daytime as well as at night their perfume is faint until after nightfall. The ripened capsules are erect. The leaves are elliptical to spatulate-oblong with a broad clasping and slightly decurrent base. They are coarsely rugose.

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EXPLANATION OF PLATES

All the plates are from photographs taken by Mr. B. F. White under the direction of W. A. Setchell.

PLATE 1

"Brazilian" Tobacco. U. C. B. G. $\frac{71}{05}$. $\times \frac{3}{32}$ diam.

71
05

PLATE 2

"Cavala" Tobacco. U. C. B. G. $\frac{72}{05}$. $\times 3/32$ diam.

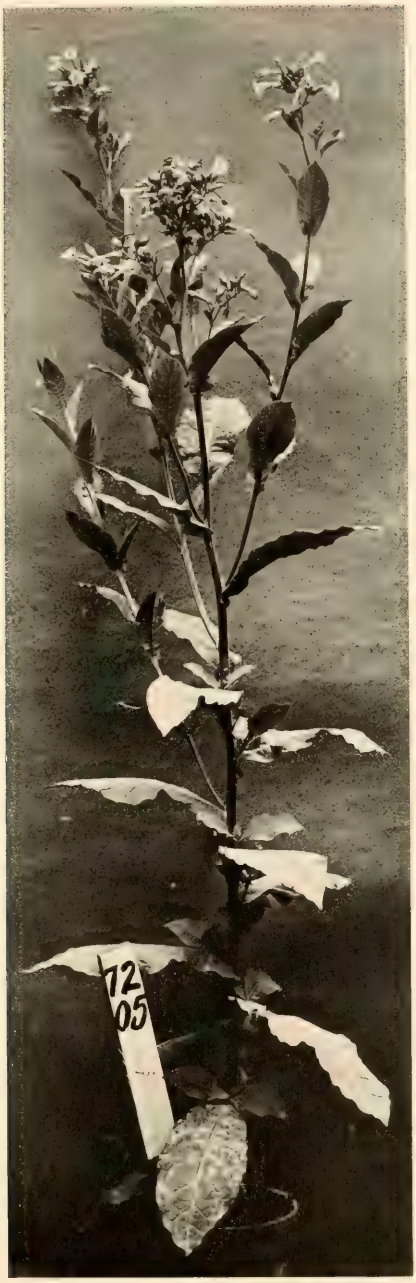
72
05

PLATE 3

"Maryland" Tobacco. U. C. B. G. $\frac{78}{05}$. $\times 7/64$ diam.

33
7850

PLATE 4

Nicotiana Tabacum var. calycina. U. C. B. G. $\frac{110}{05}$. $\times 9/64$ diam.

110
05

PLATE 5

"White Tobacco." U. C. B. G. $\frac{30}{06}$. $\times 11/128$ diam.

30
06

PLATE 6

Nicotiana Tabacum var. macrophylla. U. C. B. G. $\frac{22}{07}$. $\times 15/64$ diam.

I
22
07

PLATE 7

Nicotiana angustifolia. U. C. B. G. $\frac{68}{07}$. $\times 2\frac{1}{128}$ diam.

O
68
07

PLATE 8

Nicotiana Tabacum var. macrophylla purpurea. U. C. B. G. $\frac{25}{06}$. $\times \frac{5}{64}$ diam.

25
06

PLATE 9

Nicotiana rustica var. asiatica. U. C. B. G. $\frac{12}{07}$. $\times 21\frac{1}{12}$ diam.

O
12
07

PLATE 10

Nicotiana rustica var. brasilia. U. C. B. G. $\frac{13}{07}$. $\times \frac{1}{8}$ diam.

X
13
07

PLATE 11

Nicotiana rustica var. humilis. U. C. B. G. $\frac{14}{07}$. $\times 15/64$ diam.

X
14
07

PLATE 12

Nicotiana rustica var. jamaicensis. U. C. B. G. $\frac{15}{07}$. $\times 1\frac{1}{64}$ diam.

1/5

PLATE 13

Nicotiana rustica var. scabra. U. C. B. G. $\frac{26}{06}$. $\times 7/64$ diam.

26
Nov

PLATE 14

Nicotiana rustica var. texana. U. C. B. G. $\frac{17}{07}$. $\times 3/16$ diam.

O
17
07

PLATE 15

Nicotiana rustica var. pumila? U. C. B. G. $\frac{169}{08}$. $\times 7/16$ diam.

169
08

PLATE 16

Nicotiana rustica var. pumila ? U. C. B. G. $\frac{169}{08}$. $\times 3\frac{1}{128}$ diam.

PLATE 17

Nicotiana Langsdorffi. U. C. B. G. $\frac{102}{05}$. $\times \frac{1}{8}$ diam.

102
05

PLATE 18

Nicotiana Langsdorffii var. grandiflora? U. C. B. G. $\frac{107}{08}$. $\times 1\frac{3}{64}$ diam.

107
08

PLATE 19

Nicotiana Langsdorffii var. longiflora ? U. C. B. G. $\frac{173}{08}$. × 11/64 diam.

173
08

PLATE 20

Nicotiana paniculata. U. C. B. G. $\frac{106}{05}$. $\times 11/64$ diam.

106
05

PLATE 21

Nicotiana glutinosa. U. C. B. G. $\frac{79}{07}$. $\times \frac{1}{8}$ diam.

(The label $\frac{79}{09}$ should be $\frac{79}{07}$).

79
07

PLATE 22

Nicotiana tomentosa. U. C. B. G. $\frac{193}{08}$. $\times 1\frac{3}{64}$ diam.

(A small plant grown from a cutting).

PLATE 23

Nicotiana noctiflora? U. C. B. G. $\frac{9}{07}$. $\times 13/64$ diam.

9/07

PLATE 24

Nicotiana longiflora. U. C. B. G. $\frac{100}{05}$. $\times \frac{1}{8}$ diam.

(The flowers are closed; daytime condition).

100
05

PLATE 25

Nicotiana alata var. grandiflora. U. C. B. G. $\frac{98}{05}$. $\times \frac{3}{32}$ diam.

(The flowers are closed; daytime condition).

PLATE 26

Nicotiana acuminata var. grandiflora. U. C. B. G. $\frac{150}{07}$. $\times \frac{9}{64}$ diam

150
07

PLATE 27

Nicotiana repanda. U. C. B. G. $\frac{79}{09}$. $\times 9_{64}$ diam.

PLATE 28

Nicotiana sylvestris. U. C. B. G. $\frac{107}{01}$. $\times \frac{3}{32}$ diam.

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QUANTITATIVE STUDIES OF INHERITANCE IN *NICOTIANA* HYBRIDS *

I. THE RELATION BETWEEN THE WEIGHTS OF HYBRID TOBACCO SEED AND THE INHERITANCE OF CERTAIN CHARACTERS IN F_2.

II. QUANTITATIVE EXPRESSION OF IMPERFECT DOMINANCE IN THE COROLLA DIAMETERS OF THE FLOWERS ON THE HYBRIDS PRODUCED FROM THREE VARIETIES OF *NICOTIANA ACUMINATA*

BY

THOMAS HARPER GOODSPEED

THE RELATION BETWEEN THE WEIGHTS OF HYBRID TOBACCO SEED AND THE INHERITANCE OF CERTAIN CHARACTERS IN F_2

CONTENTS

PAGE

I. Introduction 88
II. Description of experimental material 89
(1) Distinguishing characteristics of parents and appearance of the hybrid in F_1 89
III. Division of the hybrid seed according to—
(1) Size and shape 91
(2) Color 92
(3) Weight 92

* A thesis in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the University of California.

IV. Germination of the seed 96
(1) In the germinating case 96
(2) In the propagating house 98
(3) Total germination and discussion of rates of germination. 99
V. Development of Seedlings 101
(1) Early development of seedlings 101
(2) Number and condition of plants in the field 101
VI. Appearance of the plants in the field 103
(1) Scheme of grouping 103
VII. Discussion of inheritance of characters according to weight of seed 106
(1) Shape, size and weight of parental seeds of 1906 and 1907 106
(2) Correlation between inheritance of characters and weight of seed 107
VIII. Summary of results 108
IX. Discussion of results 109

I. INTRODUCTION

The investigation herein reported was undertaken for the purpose of determining the possible relationship between the physical characteristics of pedigreed seed and the segregation of the so-called "unit characters" as manifested in the plants grown from such seed—especially the relation between the weight of hybrid tobacco seed and the appearance of the F_2 generation individuals produced therefrom. The introduction of evidence to support a strict Mendelian interpretation of experiments in plant breeding, the results of which fail to show segregation in accordance with any of the accepted formulae, has very naturally reached such a point that some interest must be attached to the amount of germination, viability, and general physical constitution of the seed from which the pedigreed plants have developed, or are to develop. Indeed, in any scientifically conducted experiment in plant breeding, there should be more attention paid to such supplementary data as the variations in color, size, weight, and germination of the seed.

There seems to be no mention throughout the literature of any recorded data in this particular connection (see, however, Groth, 1911, part I, page 9), though a great number of practical experiments, dealing with the relation between the physical characteristics of seed and the vigor, etc., of the resulting plants,

have been fully reported. Thus Clark (1904) worked upon the value of seed-selection according to specific gravity and summarizes the work, along this particular line, of previous investigators—Haberlandt, Wollny (1885), Hellriegel (1883), and others. Shamel (1904), Weber and Boykin (1907), Lill (1910), Scherffius (1909), and many others have conducted investigations connected with the relation of weight, size and density to germination and subsequent development of wheat, cotton-seed, tobacco seed, etc. The general result of all this work has been to establish the value for the agriculturist of careful seed-selection and especially the advantage of sowing heavy rather than light seed for the subsequent production of uniformally vigorous plants and thus of the most valuable crops. In agricultural practice the plants grown from heavy tobacco seed have been found to give often over 90 per cent germination and to produce plants that grew more rapidly, matured earlier and were best developed and most vigorous; while from light seed "small, irregular and undesirable" plants develop (Shamel, 1904, p. 440; cf. Waldron, 1910, and Love, 1910, p. 423). These conclusions are based upon the separation of well-known commercial strains of tobacco and give no results directly applicable to the investigation in hand.

II. DESCRIPTION OF EXPERIMENTAL MATERIAL

Distinguishing Characteristics of Parents and Appearance of Hybrids in F_1

The experimental material consisted of the second hybrid generation seed produced by self-fertilizing the flowers of one F_1 hybrid plant, representing the cross *Nicotiana Tabacum* var. *macrophylla* ♀× *Nicotiana Tabacum* var. *virginica* ♂. *Nicotiana Tabacum* var. *macrophylla* is U. C. B. G. $\frac{22}{07}$ (Setchell, 1912, p. 8, plate 6); the original seed was received under the name quoted from Professor O. Comes. *Nicotiana Tabacum* var. *virginica* is U. C. B. G. $\frac{78}{05}$ (Setchell, 1912, p. 6, plate 3), a form of *N. Tab.* var. *virginica,* Comes (1899), but is better referred to under the trade name of *Maryland,* under which name the

original seed was received from the United States Department of Agriculture. The parental types have been grown in the University of California Botanical Garden (U. C. B. G.) for five and seven years respectively under the direction of Professor W. A. Setchell and have almost from the first been propagated, in both cases, from "pure" seed in the pure line. The principal and most striking characteristics which distinguish the two types and the F_1 hybrid are summarized in the following table:

	FLOWER			HABIT		LEAF		
	Color	*Size*	*Corolla limb*	*Height*	*Width*	*Petiole*	*Tip*	*Auricle*
Nicotiana Tabacum variety *macrophylla*	magenta	large	flat, pentagonal, angles shallow	average 90 cm.	wide and branching from base	heavily winged	blunt	flat
Nicotiana Tabacum var. *virginica*	light pinkish	smaller	cut into lobes each tipped with a recurved hook	average 150 cm.	upright and not branching from base	light winged	curved long & narrow	ruffled
Hybrid between *Nicotiana Tabacum* var. *macrophylla* ♀ and *Nicotiana Tabacum* var *virginica* ♂	pink to light red	fairly large	As *N. macrophylla*, but angles not so shallow and tipped with recurved hooks	from 62 to 170 cm.	wide and branching	heavily winged	two above combined	somewhat ruffled

It can be seen from the above table that, in general, one would be justified, on the Mendelian basis, in considering the *N. Tabacum* var. *macrophylla* parent as dominant and the *N. Tabacum* var. *virginica* parent as recessive. After a general examination in the field of the F_1 generation, this dominance would have been rather striking and, for convenience of reference and later discussion, the fact that such dominance was apparent will be recognized. The fact that on close examination of the hybrids the influence of the *virginica* parent was shown in the leaf-shape and auricle, the flower-color and corolla lobes and that, among the fifty individuals grown, the height of the central axis was found to vary between 62 and 170 cm., the width of the broadest leaf from corresponding regions between 15 and 37 cm., while the length of the longest leaf varied between 28 and 59 cm., all these, in general, support the conclusion arrived at in the following communication (cf. p. 117).

III. DIVISION OF F_1 HYBRID SEED

The technique and methods in obtaining pure seed, performing the cross-pollinations and the cleaning and sowing of the seed, has been fully described elsewhere in this combined paper (pp. 126–131). The parent plants were crossed during August, 1909, and the hybrid plants produced from the seed of this cross, and which yielded the experimental material, were grown during the summer and fall of 1910.

When the seed, produced by self- (close) fertilizing one of the plants of this hybrid, was cleaned in January, 1911, there were five ripe and well-seasoned capsules in the bag. During February, 1911, the seed of this one plant was examined for size, color and weight as follows.

1. Size and Shape

Under the lens the seed presented a characteristic appearance in all instances. The shape was more or less oval to flattened pear-shape, with the funiculus often forming a little hook at the smaller end of the seed. In most cases, also, a seam was to be seen running from the short funicle for some distance around the seed soon to fade, however, into the pimply seed-covering. This pimply appearance is caused by a great number of sharply raised points occuring on the surface of the seed coat, which thus appears quite rough under a low magnification. Under a considerable magnification these raised points were seen to be connected with one another by fine, much curved and twisted, thread-like lines. Under the lens an arbitrary division was made according to size into (1) large, (2) intermediate, (3) small—all of these were well filled out, plump seed—and (4) ill-formed, flattened and wrinkled seed. The seed placed in division 4 presented a great variation in appearance, from large seed coats, so flattened as to appear empty, to very small seeds with simply much wrinkled coatings. The average length, breadth and the length-breadth index for the seed of grades 1, 2 and 3 are given in the following table:

Grade	Length	Breadth	Length-breadth index
1.	0.85 mm.	0.60 mm.	70.58
2.	0.75 mm.	0.58 mm.	77.33
3.	0.66 mm.	0.43 mm.	65.15

Measuring ten fields, indiscriminately chosen, under the low power of the dissecting microscope and including in each field from 90 to 115 seeds, the proportion of the various grades per field was as follows:

Grade 1.....26 %
Grade 2.....46 %
Grade 3.....22 %
Grade 4..... 6 %

The separation of seed according to size was combined with the separation according to weight and will be taken up in this connection later on.

2. Color

In color there were practically no variations from the typical very dark brown coloration of the seed coats. A few seeds at times seemed to present a somewhat lighter appearance, but their number was so small and the normal color so often found to be present on some one of the other faces of the seed, that no separation according to color was attempted.

3. Weight

For the separation of the various weights of seed an improvised "grader," on the general style of that described in the work on wheat separation in the Kansas Agricultural Experiment Station (Lill, 1910), was employed for the first step in the process. A large centrifugal fan on a 110-volt alternating current supplied a constant draft of air which was caught in a large paper funnel and conveyed through a three-foot section of glass tubing, one inch in diameter. The tube was tilted upward from the source of the air-current at an angle of fifteen degrees and the seed introduced through a tin funnel at its lower end. The proper point, near the lower end of the tube, at which

the seed could be poured in and neither be driven back up the tin funnel by the air-current nor fall back down the tube into the pan, was determined by experiment, as was also the proper angle at which the tube should be tilted to give the necessary "throw" to the seed after it left the upper end. A large paper shield, reaching to the table, was fastened two inches back of the upper end of the tube and thus effectually excluded any current of air in the region through which the seed fell and about the receiving boxes. These boxes, eight of them and placed end to end, extended for three feet from a point directly below the outlet of the tube. A box with high, sharply sloping sides, partially closed at the extreme end and open above the receiving boxes, extended the full length of the boxes to catch any scattering seed and direct it downward. The maximum drop of the seed into the boxes from the mouth of the tube or during its progress through the air above the boxes, was about twenty inches, though by far the majority of the seed began to fall, to some extent, as soon as it was free from the tube.

After considerable effort had been expended in perfecting the arrangements and determining by direct experiment the proper position for the receiving boxes, etc., it was possible to accomplish the entire grading of the seed on two successive days during approximately the same hours. Since a motor attachment was not available, it was not possible to regulate the speed of the fan exactly and thus secure a perfectly constant blast of air for any length of time. But by using the apparatus on two successive days and at the same hours, it was judged and proven by actual test that the blast was approximately the same during the whole time of grading the seed.

The seed was slowly poured into the funnel directly from the packet into which it had been cleaned until perhaps a thousand seeds had fallen into the receiving boxes. This first grading was always very rough and gave only an approximate separation according to density. Contrary to expectation, it was found that the heaviest seed continually fell in the boxes furthest from the opening of the tube, while the lightest and medium seed fell into the first five boxes. The ill-formed, chaffy seed was distributed throughout the whole of the eight boxes,

probably because of the inequality of the surfaces which the seed presented to the air-blast. The seed that had fallen into the first three boxes—nearest the opening of the tube—was then put into the grader a second and a third time, at the end of which the second box was found to contain the majority of the seed. The seed from this second box was then run through the apparatus three or four times more and, at the end, fifty to sixty seeds remained. By marking certain of the seeds it was found that they continually fell during each of the six or seven gradings into the same receiving box or, rarely, struck the edge to fall into the box just before. The same method was employed in the combined boxes four and five and the majority of the seed was at the end found in box five. Similarly for the heaviest seed in boxes six, seven and eight the seed at the end was taken from box seven. After twenty-one gradings, approximately two hundred seeds of three grades had altogether been obtained. The remaining seed in the boxes was then mixed and, after two or three hundred seeds from the original packet had been added, the whole process was repeated. Over two hundred and seventy-five gradings were made and, in all, twenty-seven hundred seeds were separated out into grades.

All the seed was then examined under a low-power magnifying glass—i.e., a reading-glass lens four inches in diameter, with a focus of nine inches. The extremely small size of tobacco seed makes any such examination a relatively slow and tiresome process. The seed was moved about under the lens on a glass plate with a fine-pointed camels-hair brush. The removal of the ill-formed, chaffy seed that was found in all the grades was the first object of this examination and some 350 individuals of this type were picked out from the whole 2700 seeds examined. In the heavy seed a remarkable uniformity in size and shape was noted, though there were among the 1100 heavy seeds examined some thirty markedly smaller seeds which were arbitrarily discarded. In all such cases the discarded seed was thrown away and not used in making up other groups of seed. The examination of the medium seed, which had fallen into the intermediate boxes four and five, showed a considerable variation in size. All seed that approximated the size noted

for the heavy grades was discarded and also the very minute seeds noted above as having occurred among the heavy grades—64 seeds in the first case and 80 seeds in the second, out of 400 seeds examined. The remaining seed was fairly uniform in size and distinctly smaller than the heavy seed. Among the light seed, which had been taken from box two, the greatest number of ill-formed seed occurred and also twenty per cent of the minute seed—this on the basis of twelve hundred seeds examined. It was found possible to separate out these very small seeds and still have a light grade of seed that was distinctly set apart from the medium seed in size and was about one-half the size of the heavy grade. From the various grades and the ill-formed seed a total of 2200 seeds, in separate packets of 100 each, were counted out preparatory to weighing.

The scales used were the mechanical balances manufactured by Josef Nemetz of Vienna and weighing from 0.0001 of a gram to 1 gram only. Since more delicate balances were not available, it was necessary to weigh the extremely small tobacco seed by hundreds and then by tens. The following table gives the weights of the various grades of seed, as divided by the air-blast, in terms of 100 seeds for each number and thus really expresses the average weight of groups of 100 seeds from each grade.

Ill-formed seed	Light seed	Medium seed	Heavy seed
1. 0.0042	3. 0.0044	7. 0.0063	13. 0.0086
2. 0.0041	4. 0.0057	8. 0.0064	14. 0.0091
	5. 0.0059	9. 0.0065	15. 0.0094
	6. 0.0061	10. 0.0067	16. 0.0095
		11. 0.0070	17. 0.0095
		12. 0.0073	18. 0.0097
			19. 0.0098
			20. 0.0099
			21. 0.0099
			22. 0.0111

In each case the 100 seeds were counted into the pan of the balances—picking up each seed with a brush—weighed, then removed and weighed again by tens. The weights of the groups of ten seeds were, with the exceptions noted below, in every case found to be so nearly the same that it was felt, by ex-

pressing the weights of the seeds in terms of 100 individuals, a true average weight per seed could be calculated. For the same reason it was felt that few individuals, if any, weighed much more or less than this average or, in other words, that every one of the hundred seeds of group 9, for example, which weighed 0.0065 grams, corresponded, more or less closely, to a theoretical weight of 0.000065 grams. The above is also, in effect, a combined table expressing the various sizes and densities of the different groups of seeds, as well as their weight, since all three characters were found to be so closely correlated as mutually to represent one another. Groups 1 and 2, the ill-formed seed, showed naturally a considerable difference in weight among the groups of ten seeds, since they included all abnormally shaped seed, from those that were simply shrunken and wrinkled to those whose seed-coats were flattened and practically empty. The minute seed, that was picked out in the final examination after grading from the group of least density, is included in 3.

IV. GERMINATION OF THE SEED

1. In the Germinating Case

For germinating the seeds it was necessary to use an improvised germinating case. This was made by heavily insulating a large glass-sided box with herbarium blotters and using a 16-candle power electric light, immersed in water, to obtain the desired temperature within the box. It was so arranged that the germinating case could be entirely closed after the seed had been put in and the water about the electric light renewed through a tube. The jar of water in which the light was placed was tightly surrounded by a blotter and the opening at the top of the jar was covered with a zinc plate so that practically no light could be seen within the box. The blotting-paper insulation was left free on one side and could be removed when observations of the progress of germination were to be taken. The seeds were counted out from the packets in which they had been placed after weighing onto circular pieces of blotting-paper

so cut that their diameter was a little greater than that of an ordinary drinking-glass. These circular pieces of blotting-paper were placed upon glasses nearly filled with tap water and a connection was so made with strips of blotting-paper reaching down into the water that the seed-holders were kept continually moist without allowing an excess of water to collect about the seeds themselves. A watch crystal covered each group of 100 seeds and their seed-holder and served to keep the drops of moisture which condensed on the top of the germinating box from falling onto the seeds and also aided in maintaining a constant temperature about the seed (see Garman, 1910, p. 44). The temperature was very fairly constant within the box, at 21° C., and never varied over three degrees throughout the first twenty-six days of the experiment. The germinating case was sterilized as thoroughly as possible and the seeds, arranged as above described, placed within it on February 28. The following table gives the extent of germination between March 3 and March 31 for the different weights of seed. The numbers given in the first vertical column refer to those used in the table on page 95 to designate the 22 different weights of seed.

	March	3	5	7	9	10	11	12	13	14	16	17	18	20	21	23	27	29	31	%	
Light seed	1 & 2	9	8	7	1	..	..	1	..	..	1	1	..	..	2	..	..	..	..	16	Average 66%
	3	6	9	5	8	..	..	..	..	..	6	..	..	4	..	1	..	3	..	42	
	4	16	27	12	2	2	4	2	..	..	4	..	1	..	3	..	..	..	..	73	
	5	*	40	8	6	1	2	1	5	..	..	..	..	..	4	..	..	..	..	67	
	6	20	29	..	8	2	5	4	..	..	6	2	1	2	3	..	..	..	..	82	
Medium seed	7	*	44	..	3	..	5	1	..	..	3	4	..	3	6	..	2	..	..	71	Average 64%
	8	12	18	10	..	..	4	1	3	..	4	..	1	1	5	..	..	..	..	59	
	9	*	45	6	2	1	..	3	5	..	4	..	1	2	6	..	1	..	..	76	
	10	*	41	4	4	1	..	..	6	2	..	..	1	..	..	..	..	..	..	59	
	11	*	44	3	4	3	3	2	..	2	..	..	..	..	5	..	..	..	..	66	
	12	*	39	..	4	2	1	2	2	..	..	..	1	1	2	..	1	..	..	55	
Heavy seed	13	10	15	4	6	..	..	..	..	7	..	4	3	..	..	7	4	..	3	63	Average 49%
	14	4	4	4	..	..	1	1	4	1	3	2	6	..	8	..	..	..	..	38	
	15	6	11	10	4	6	5	6	..	3	6	6	3	..	1	..	1	..	..	68	
	16	7	11	..	7	..	1	..	..	..	..	6	..	..	3	..	..	8	3	47	
	17	3	..	12	9	3	..	3	..	..	4	..	2	4	12	..	3	..	..	55	
	18	1	..	12	6	5	6	4	2	..	..	..	1	5	7	4	..	..	..	53	
	19	6	8	6	5	6	..	..	5	..	3	..	2	3	11	5	2	1	1	64	
	20	2	4	..	4	..	3	2	7	..	4	..	1	3	..	3	..	..	..	33	
	21	6	11	..	3	2	1	2	..	3	..	..	..	3	2	..	4	..	..	37	
	22	6	..	2	10	..	5	1	3	..	..	..	2	..	5	..	1	..	..	35	

* Over 25 had germinated but the number was not counted on this date.

When germination was first noted on March 3, the numbers given above under that date express the number of seeds from which, in each case, the white caulicle was protruding and the cotyledons also were beginning to appear. This condition of the seeds was throughout taken as the criterion of germination. The germinating case was not opened until March 10, at which date many of the seedlings were entirely free from the seed coats, the greenish cotyledons were fully expanded and considerable growth of the first rootlets had taken place. On this date and on March 13, 16, 20, 27, and 31, all the seeds were removed from the case and all the seedlings, free from the seed coats, were planted in four-inch pots of sterilized soil and each pot covered with a glass plate. The pots were watered from beneath and placed near an east window.

Though no difficulty, except in the case of the ill-formed, chaffy seed, was experienced in connection with moulds attacking the seed in the germinating case, the pots containing the seedlings were much affected and quite a number of the young plants damped off during the first three weeks, because of the thick layer of mould over the surface of the soil.

2. In the Propagating House

Since germination had been very slow throughout all the weights of seed between March 21 and 31, it was thought best to change the conditions under which germination had been taking place. To this end, on April 1, the remaining seeds were picked off from the blotting-papers and lightly sown in sterilized soil. The pots containing the seed that had not germinated were then removed to a propagating house in which temperature and moisture conditions suffered daily a wide degree of variation. No sign of germination was observed among any of the weights of seed for six days and then only a slight germination among the light-weight seed. When it was next possible to note the germination—i.e., April 28—extensive germination was found to have taken place, especially among the heavy weights of seed, as noted below.

	Numbers of seed weights	Number of seed germinated between April 1 & 31	Total percent of germination	Average percent of total germination of grades of seed
Ill-formed seed	1 & 2	0	16	16
Light seed	3	10	52	76
	4	13	86	
	5	12	79	
	6	5	87	
Medium seed	7	16	77	80
	8	23	82	
	9	13	89	
	10	21	80	
	11	20	84	
	12	18	73	
Heavy seed	13	26	89	88
	14	47	85	
	15	22	88	
	16	39	86	
	17	25	79	
	18	31	84	
	19	24	88	
	20	54	87	
	21	56	83	
	22	62	97	

3. Total Germination and Discussion of Rates of Germination

Under constant experimental conditions over 50 per cent of the light-weight and medium-weight seeds germinated in less than four weeks. Under the same conditions and during the same period of time 40 per cent of the heavy-weight seeds germinated (Shamel & Cobey, 1907, p. 58).

There was fairly regular diminution in the amount of germination under these constant experimental conditions and during this month, as the weight of the seeds increased.

Under widely varying conditions of temperature and light, the heavy-weight seed which had not germinated in the germinating case gave an average germination of 38 per cent in four weeks. Under these widely varying conditions and during this period of time the light and medium weights of seed gave an average germination of 18 per cent.

The final count, which includes germination in the germin-

ating case and in the propagating house, showed a fairly regular increase in total germination, beginning with the lightest grades of seed and running up to the heaviest.

The results of numerous investigations on the delayed germination of seeds and the water and oxygen requirements for germination, point out the probable causes of the early germination of the light seed and the delayed germination of the heavy seed (Shull, C. A., 1909 and 1911, and the literature there cited). Thus (1) the seed-coverings of the majority of the light seed may be more permeable to water and oxygen than those of the heavy seed, or (2) the water and oxygen requirements for the germination of the heavy and light seeds may not be the same and, (3) the oxygen content of the medium surrounding the seeds in the germinating case and in the propagating house was certainly higher in the latter situation. Thus, again, with a high permeability for water and oxygen in the seed coats and a low oxygen requirement for the germination of the light seed, we should find this light seed showing high average germination in the germinating case. On the other hand, with slight permeability of the seed coats of the heavy seeds for water and oxygen and a high oxygen requirement for their germination, we might expect to find the average germination in the germinating case relatively low and that rapid increase in germination would sooner or later be apparent with the increased oxygen about the seed in the propagating house. The conclusions of various investigators (Strasburger, Noll, etc., p. 258) that variations in temperature increase germination may also give a suggestion as to the cause of the high average germination of the heavy seeds in the propagating house (see also Raciborski, 1900).

V. DEVELOPMENT OF SEEDLINGS

1. Early Development of Seedlings

Since there was space available for growing only 230 plants of the hybrid *N. Tabacum* var. *macrophylla* ♀× *N. Tabacum* var. *virginica* ♂, twenty-five of the developing seedlings only of each weight of seed were retained. Each group of twenty-five, with the exception of numbers 1 and 2, from which only ten plants were available, was composed in practically every case of seedlings of all ages, i.e., those developed from seed which had germinated in the germinating case and those produced from seed which had germinated after April 1 in the propagating house.

The growth and early development of all the seedlings was, in general, normal and corresponding in every way. As the seedlings were passing into the typical semi-rosette stage, it was observed that the plants grown from heavy seed were developing more rapidly than those grown from the light seed, though these last had in general germinated a month earlier. This difference in size and general development on May 18 is shown in plate 34, figure 1. As can be seen, the difference in size of the two groups of plants was mainly due to retarded development of the plants from the light seed or, on the other hand, to abnormally rapid growth of the plants from the heavy seed, and was not to any great extent due to a weak or sickly condition of the former.

2. Number and Condition of Plants in the Field

The plants were set out in the field early in July. They were placed in two long rows of approximately 110 plants each, with the plants 3½ ft. apart in the rows and with ten plants representing each number given in the table of the various weights of the seed. The following table gives the number of normal and vigorous plants growing in the field when first carefully examined on September 2:

	Weights of seed Numbers of	Number of plants in field Sept. 2	% of plants, originally in each number, growing on Sept. 2
Ill-formed seed	1 & 2	8	80
Light seed	3	7	87
	4	10	
	5	8	
	6	10	
Medium seed	7	10	86
	8	6	
	9	10	
	10	10	
	11	8	
	12	8	
Heavy seed	13	7	82
	14	10	
	15	10	
	16	8	
	17	7	
	18	8	
	19	10	
	20	8	
	21	8	
	22	7	

From the above it will be seen that the number of plants grown from the ill-formed seed, from the light seed, from the medium seed and from the heavy seed, that came to maturity as fully developed, vigorous plants, was practically the same. This is especially evident when we consider the fact that the averages given above are based upon such a small original number as ten plants set out in the field. I am told that there were as many surviving seedlings ready to be set out in the field from the light and medium weights of seed as from the heavy seed. On September 2 these plants had formed over twenty capsules of seed apiece, were or just had been fully in flower and were, in most cases, making vigorous laterals on which numerous flower buds were forming.

VI. APPEARANCE OF PLANTS IN THE FIELD

1. Scheme for Grouping Plants on the Basis of the Combinations of Characters which they Exhibited

Between September 18 and 25, all the plants were carefully gone over, separately examined, and the exact appearance of each plant was noted under the following headings:

I. Flower
1. Color
   (a) pinkish
   (b) pink
   (c) magenta
2. Corolla limb.
   (a) flat, pentagonal, angles shallow
   (b) cut into lobes tipped with a recurved hook

II. Habit
1. Tall
2. Short
3. Spreading
4. Not spreading

III. Leaf
1. General shape
   (a) elongated lanceolate
   (b) ovate subrotund
   (c) lanceolate subrotund
2. Leaf tip
   (a) Slender-pointed and curving
   (b) sharp pointed and not as (a)
3. Auricle
   (a) flat
   (b) ruffled.

On the basis of such a tabulation of characters, a plant which reproduced the *N. Tabacum* var. *virginica* parent in appearance would have been noted as follows:

Flower color—Pinkish.

Shape of corolla limb—Cut into lobes each tipped with a recurved hook.

Habit—Tall and with no laterals broadly spreading from the base.

Leaf shape—Elongated lanceolate with the tips of the leaves slender-pointed and curving and the auricle ruffled.

Similarly for a plant reproducng the *N. Tabacum* var. *macrophylla* parent in appearance:

Flower color—Red.

Corolla limb—Pentagonal with the angles shallow and not hooked.

Habit—Short, with laterals widely spreading from the base, and the

Leaf—ovate subrotund, with a short, almost blunt tip and a flat auricle.

There was not a single individual which corresponded to either of these two descriptions.

A plant that could be spoken of as "intermediate" would have exhibited the

Flower color—Pink.

Corolla limb—Pentagonal with angles elongated into short hooks.

Habit—Tall, with numerous laterals spreading from the base, and

Leaf—Lanceolate subrotund, with a long, curved, slender tip and a somewhat ruffled auricle.

A plant said to "resemble" one or the other parent would show some three or four of its minor characteristics differing from, but otherwise nearly identical with, the parent the appearance of which it most nearly approximated—i.e., approaching the typical appearance of one of the parents as nearly as the F_1 (so-called) dominant approximated the appearance of the *N. Tabacum* var. *macrophylla* parent. Finally, the plant called a "blend" would show a typical flower of one parent combined with an "intermediate" leaf-shape and the habit of the other parent, or any other of the possible combinations in which one or two characters each, of both parents appears fully developed in the hybrid.

2. Number of Plants in Various Groups

The following table gives the number of plants from each weight of seed and the combination of "characters" which they exhibited. The meaning of the terms "intermediate," "resembling" and "blend" have been described above.

	Numbers of weights of seed	Inter mediates	Resembling *N. Tabacum* var. *macrophylla*	Resembling *N. Tabacum* var. *virginica*	Blends
Ill-formed seed	1 & 2	2	2	2	2
	3	1	1	2	1
Light seed	4	2	2	3	3
	5	2	1	2	3
	6	1	2	4	3
Medium seed	7	1	2	3	4
	8	1	2	1	2
	9	2	2	3	3
	10	2	4	1	3
	11	1	2	3	2
	12	1	2	2	3
Heavy seed	13	2	1	1	3
	14	4	4	0	2
	15	1	4	1	4
	16	1	3	0	2
	17	2	3	0	2
	18	3	2	2	3
	19	1	2	2	3
	20	1	4	2	1
	21	2	5	0	1
	22	2	3	0	2
	Heavy seed	19 (24 %)	31 (39 %)	7 (9 %)	22 (28 %)
	Medium seed	8 (15 %)	14 (26 %)	13 (25 %)	17 (33 %)
	Light seed	6 (18 %)	6 (18 %)	11 (33 %)	10 (31 %)

No possible statement in tabulated form or otherwise could be included in a paper of this scope which would give any accurate summary of the multitude of combinations of the parental characters in the most widely varying degrees which the 175 F_2 generation plants exhibited. Indeed no two individual judgments on any one plant would coincide except in a general way. Thus, for the table given above, there can be claimed no absolute degree of accuracy. The main results as given, however, were certainly present in a rather marked degree. These results seem to indicate that some correspondence exists between the various weights of first generation hybrid tobacco seed and the appearance of the F_2 generation plants produced from the various divisions of this seed according to weight.

VII. DISCUSSION OF INHERITANCE OF CHARACTERS ACCORDING TO WEIGHT OF SEED

1. Shape, Size and Weight of Parental Seed of 1906 and 1907.

The seed which was used to reproduce *N. Tabacum* var. *macrophylla* and *N. Tabacum* var. *virginica* in 1907 and 1908 was examined under the lens and also weighed. There was under the reading-lens a greater uniformity in shape and size of the seed of both parents than that which has above been noted in reference to the shape and size of the F_1 hybrid seed produced by the cross between these plants. The average length and breadth and the length-breadth index of the seed *N. Tabacum* var. *virginica* and the seed of *N. Tabacum* var. *macrophylla* harvested in 1907 is given in the following table.

N. Tabacum var. *virginica*			*N. Tabacum* var. *macrophylla*		
Length	Breadth	Length-breadth index	Length	Breadth	Length-breadth index
0.76 mm.	0.55 mm.	72.48	0.88 mm.	0.61 mm.	69.31

The average weight of 200 seeds of *N. Tabacum* var. *virginica* harvested in 1906 was only 0.0001 gr. less than the average weight of 200 *N. Tabacum* var. *virginica* harvested in 1907.

400 *N. Tabacum* var. *virginica* seed harvested in 1906 and 1907 weighed (average for 100 seeds), 0.0069.

200 *N. Tabacum* var. *macrophylla* seed harvested in 1907 weighed (average for 100 seeds), 0.0078.

These 600 parental seeds were weighed just as before described in connection with the weighing of the F_1 generation hybrid seed. In picking out the seed to be weighed, no effort was made to make any distinction between seeds of slightly different sizes and shapes—i.e., the average run of seed was taken except that the small number of ill-formed, chaffy seed that occurred was not included.

2. Seeming Correlation between Weights of Seed and Inheritance of "Dominant" and "Recessive" Characteristics

The fact that the "dominant" parent—*N. Tabacum* var.*macrophylla*—when self- (close) fertilized in 1907 produced seed which was heavier than the seed produced in the same manner by the "recessive" parent—*N. Tabacum* var. *virginica*—in both 1906 and 1907, seems to be significant when we consider the weights of the seed produced by the cross between these two parents and when we consider the appearance of the F_2 generation produced from the various weights of the hybrid seed. For the heavy portion of the seed produced by self-fertilizing the hybrid between *macrophylla* and *virginica* gave F_2 hybrid plants of which 39 per cent more closely resembled the *macrophylla* parent than they did the *virginica* parent. Secondly, among the plants produced by this heavy hybrid seed only 9 per cent resembled *virginica*. Again, from among the lighter weights of the F_1 generation hybrid seed there were produced, as F_2 generation hybrids, 33 per cent of plants resembling *virginica* as against 18 per cent resembling *macrophylla*. Finally, from medium weights of hybrid seed there were produced approximately the same percentage of plants resembling *macrophylla* and resembling *virginica*.

Thus the parent of the cross *N. Tabacum* var. *macrophylla* ♀× *N. Tabacum* var. *virginica* ♂ which possessed the characteristic of bearing heavy seed is reproduced, more or less intact, in the F_2 generation grown from the heavy weights of the F_1 hybrid seed. Thus, again, the parent of the above cross which is distinguished by bearing relatively light seed likewise appears, more or less intact, in the F_2 generation and is produced therein from the lighter weights of F_1 generation hybrid seeds. Finally, the medium weights of F_1 hybrid seeds gave plants in the F_2 generation approximately 50 per cent of which resembled *N. Tabacum* var. *macrophylla* and 50 per cent resembled *N. Tabacum* var. *virginica* in appearance.

VIII. SUMMARY OF RESULTS

1. The hybrid in F_1 produced from the cross *Nicotiana Tabacum* var. *macrophylla* ♀× *N. Tabacum* var. *virginica* ♂ resembles more closely the *macrophylla* parent.

2. The absence of complete dominance of the *macrophylla* parent was shown by the occurrence in the F_1 heterozygote of the ruffled auricle, the hooks terminating the shallow angles of the pentagonal corolla limb, the lighter color of the flowers and the more gradually tapering points of the leaves; all characteristic of *virginica*.

3. The seeds produced by close fertilizing one F_1 hybrid plant of the cross *N. Tabacum* var. *macrophylla* ♀× *N. Tabacum* var. *virginica* ♂ showed a great variation in size and in weight.

4. The divisions according to size, density and weight corresponded closely—i.e., large seeds showed highest specific density and were the heaviest, etc.

5. Of the light and medium seed 65 per cent germinated in the germinating case within a month, while only 49 per cent of the heavy seed germinated during the same period and under the same conditions.

6. During a month in an unheated propagating house the heavy seed germinated to such an extent that the final count for the two months' germination gave 88 per cent as the average of 1000 heavy seed germinated, while the light and medium divisions of seed in the propagating house gave such a low percentage of germination that the total per cent of germination of 1000 light and medium seeds for the two months was only 78 per cent.

7. The number of plants in the field four months later showed that a larger percentage of the seedlings set out into the field from the light and medium grades of seed had come to normal maturity than from the heavy seed.

8. The appearance of the F_2 generation individuals made it possible to distinguish four classes of plants, the division being based upon the combinations of the distinguishing characters of the two parents which they exhibited.

9. From the heavy seed 39 per cent of "dominants" (resembling *macrophylla*), 9 per cent of "recessives" (resembling *virginica*) and 52 per cent of "intermediates" and "blends" could be distinguished.

10. From the medium-weight seed 26 per cent of "dominants," 25 per cent of "recessives" and 49 per cent of "blends" and "intermediates" can be reported.

11. From the light seed 18 per cent of "dominants," 33 per cent of "recessives" and 49 per cent of "blends" and "intermediates" could be recognized.

12. The seed produced by close fertilizing one plant of *N. Tabacum* var. *macrophylla,* which was harvested in 1907, showed under a reading-lens a much greater degree of uniformity in shape and size than did the F_1 hybrid seed similarly examined. The same was true for the seed of *N. Tabacum* var. *virginica* similarly produced on two successive years—1906 and 1907.

13. The average weight of 100 *virginica* seeds of 1906 was 0.0001 grams less than the average weight of 100 *virginica* seeds of 1907.

14. The average weight of 100 *virginica* seeds of 1906 or 1907 was 0.009 grams less than the average weight of 100 *macrophylla* seeds of 1907.

IX. DISCUSSION OF RESULTS

The results obtained when the rate of germination of seeds of different weights, under the germinating conditions described above, was compared, are interesting and of some significance. As has been mentioned above, the differing degrees of permeability to water and oxygen probably explains the early germination of most of the light seed that did germinate at all and the slow germination of the heavy seed. The seed of 1906 and 1907 of *N. Tabacum* var. *virginica* and of 1907 *N. Tabacum* var. *macrophylla* has, within the past month, been germinated in an unheated propagating house. One hundred seeds of each year and of each variety were used. The *macrophylla* seed showed the first signs of germination within nine days and at the end of sixteen days 89 per cent had germinated. The germination of

the 1906 and 1907 *virginica* seed was somewhat slower. At the end of nine days there was no sign of germination and after sixteen days 68 per cent of the 1906 and 71 per cent of the 1907 seed had germinated. The total germination after 20 days showed that

1906—*virginica* seed germinated to the extent of.....81 %
1907—*virginica* seed germinated to the extent of.....88 %
1907—*macrophylla* seed germinated to the extent of.....91 %

The appearance of mould about the seed that had not germinated necessitated a termination of the experiment at the end of twenty days.

This parental seed was, as before noted, indiscriminately chosen and showed throughout a marked uniformity in size and shape of individual seeds. That within less than three weeks such a high percentage of germination should have taken place among the relatively old parental seed (Shamel and Cobey, 1906, p. 35, Hayes, 1912, p. 3) is interesting and also somewhat unexpected when we recollect the results obtained in germinating the hybrid F_1 seed.

That the parental seed possesses a considerably greater degree of uniformity in shape, size and *weight* than does the hybrid seed is, in general, shown by its appearance under the lens, in particular by the close agreement between the weights of various samples of the seed, and finally by the fact that its germination was fairly simultaneous within each group. In other words, there seems to be sufficient evidence to warrant the statement that for the parental seed there is no such distinct and well-marked division into grades according to physical characteristics as was found in the hybrid seed of a plant produced by crossing these parents.

The facts of greatest significance in connection with the germination of the different divisions of F_1 hybrid tobacco seed according to weight are (1) that the seedlings in the rosette stage—i.e., sufficiently developed to be set out in the field—showed a superiority of vigor for the young plants grown from heavy seed over those grown from light seed, and (2) that at the period of maturity the number of plants fully grown and normally developed which had been produced from light seed was

as great as or greater than the number produced from heavy seed. Conclusion 1 is in accord with the generally observed facts, while conclusion 2 is distinctly opposed to the report sent out by those who, in agricultural practice, have matured plants grown from heavy and from light weights of seed. The point of greatest interest, however, lies in the fact that, if there had been no careful division of the seed according to weight and if there had been no especial interest in growing separately the plants developed from heavy and light seed, the appearance of the seedlings at the time of planting out would have led a plant-breeder to choose his further experimental material largely from seedlings grown from heavy seed, since they would be the most vigorous and would be expected to produce more normal plants than would the backward seedlings from the light seed. In the light of the experimental results reported in the foregoing pages, and even without further confirmation of these results, it seems advisable to urge upon those experimenting in plant-breeding (1) the devoting of a greater measure of attention to the physical characteristics of their pedigreed seed, (2) the making of every effort to germinate all types and grades of their seed, and finally (3) the bringing to development, in so far as practicable, seedlings which show all degrees of vigor and development at the time when they are placed in the field. That the lightest weight and even chaffy, misshapen seed will germinate under the proper conditions has been shown and, of still more significance, it has been demonstrated that seedlings produced from such seed, though backward in appearance, may ultimately come to vigorous and normal maturity (see, in this connection, Harris, J. A., 1912 *b*).

From the appearance of the F_1 heterozygote we seem, as before stated, to be dealing with a case of Mendelian dominance to the extent that the influence of the *N. Tabacum* var. *macrophylla* parent predominates in the F_1 hybrid. From the appearance of the F_2 hybrid plants we find a certain indication of segregation, but that we are dealing with a case of Mendelian segregation cannot, in the present instance, be demonstrated. It hardly appears possible, even with the extent to which factorial analysis has recently been carried and the number of Mendelian

ratios and interpretations which have been advanced, that the results obtained upon tabulating the combinations of characters exhibited by the F_2 generation plants are susceptible of a Mendelian interpretation. As is noted in the following, however, the small number of plants grown may be the cause of the situation. Granting that this is the cause, we may briefly set down the situation we should encounter upon endeavoring to arrange our experimental data according to a Mendelian scheme of analysis. The two parental forms differ from one another in respect to at least seven definite pairs of characters. The hybrid in F_1, which results from a cross between the two parents, exhibits an approximately complete dominance for one parent. There was, however, no difficulty in distinguishing between the heterozygote individuals and the plants of the dominant parent. To observe among the F_2 generation plants even an approximation of the expected Mendelian ratios, at least 15,000 plants would not only have to be grown but would need to be critically examined in order that they might be arranged into the more than 2000 possible combinations that might appear. Making our calculations along this line we should not be surprised to find that among the 175 plants grown in this experiment not a single true homozygote could be found. It has, I think, elsewhere been noted that the ordinary plant-breeder, without unlimited field space and a number of trained assistants, is forced to group the various appearances of the individuals which constitute his limited F_2, F_3, etc., generations under general headings, when he attempts an investigation of the inheritance of a polyhybrid character in which the power of n is greater than 3 or 4. As has been seen, such a method of grouping has been adopted in this experiment (see Baur, 1911, p. 213). Certain plants in the F_2 generation resembled, usually rather closely, the general appearance of one parent or the other, while many other plants produced a typical flower of one parent along with a typical leaf of the other parent, or appeared to possess flowers and leaves in which the distinguishing characters of both parents were hopelessly mingled. Thus we have classified the plants in F_2 as "resembling" one parent or the other, as "intermediates" or as "blends." We certainly have no basis for either definitely affirming or denying

the presence of a Mendelian ratio within our limited experimental material, and the following brief discussion in no way attempts to exclude the possibility of such an interpretation. Our effort is primarily to ally ourselves with those who feel that "what is urgently needed is an accurate description of the various ways in which the characters of domesticated animals and plants are inherited. It will be time enough to interpret them when our knowledge of them is a great deal more perfect than it is at present" (Darbishire, 1911, p. 240).

The experimental results in general seem capable of explanation on the assumption that we are dealing here, in the case of the seed produced by the F_1 hybrid, with an infinite series. By growing plants from the two extremes, the heavy and the light seed, we have produced a high percentage of plants resembling the two parents—one parent from the heavy seed and one parent from the light seed. Again, by using the medium weights of seed we produce 50 per cent of plants intermediate in appearance and 25 per cent each of plants resembling each of the two parents. The fact that approximately 50 per cent of the plants from each of the two extremes were "intermediate" or "blends" may be due to the inaccuracy of the weighing or to the small number of F_2 generation plants grown. In other words, had it been possible to weigh each seed separately or to grow 1000 F_2 plants, the percentage of plants from heavy seed resembling *N. Tabacum* var. *macrophylla* and of plants from light seed resembling *N. Tabacum* var. *virginica,* might have been far higher.

From another point of view, the occurrence in the F_1 hybrid seed of three readily distinguishable divisions according to weight and size is significant, and especially so when we find a rather marked uniformity in weight and in size among the seeds of both parents. The possibility suggests itself that whatever segregation there may be has taken place in such a manner within the ovary of the F_1 hybrid that it (the segregation) becomes apparent only in the physical characteristics of the seed which the F_1 hybrid plant produces. Thus on close fertilization of the F_1 hybrid plants of the cross *N. Tabacum* var. *macrophylla* ♀× *N. Tabacum* var. *virginica* ♂—these two parents producing seed of different

weights, but seed which is uniform in size and in weight for each parent—we obtain from the matured ovaries seed approximately ¼ of which is heavy, ¼ of which is light, and ½ of which is intermediate in weight between the heavy and light seed. Now upon growing the F_2 plants from the heavy weights of this F_1 hybrid seed, we obtain 31 plants, the appearance of which approximates that of the *macrophylla* parent as against seven plants which resemble *virginica*. Among the F_2 individuals produced by the light seed, eleven plants resemble *virginica* and six plants resemble *macrophylla*. Finally, from the seed intermediate in weight, approximately 25 per cent of the F_2 plants resemble one parent and 25 per cent the other parent. Thus the fact of segregation, to the extent to which it has taken place, becomes apparent when the F_2 plants are grown, in that for the heavy seed one parent is "dominant," for the light seed the other parent is "dominant" and from among the medium weight seed 50 per cent each of the two parents are produced. In addition the "dominance" seems to follow the weights of the parental seed in each case, since the heavy-seeded parent appears in F_2 in greatest number among the plants grown from heavy seed and the light-seeded parent from the light F_1 hybrid seed (see, in this connection, Waldron, 1910, p. 56; also Harris, J. A., 1912 *a*).

In this connection the following suggestion is tentatively advanced. There may be such an organization of the developing pollen from the anthers of the hybrid in F_1 that the generative nucleus in each mature pollen grain bears one of two influences. The first constitutes a "determiner" (Davenport, '08), functioning to produce the outward appearance of the *macrophylla* parent. The second influence may be one which functions for the production of the outward characteristics which distinguish the *virginica* parent. The tube nucleus may, likewise, bear any one of two influences. The first is one which functions for the production of heavy seed, after its union with the fusion nucleus in the embryo sac and the second functions for the production of light seed. Corresponding conditions may be present within the embryo sac in the case of the egg nucleus and in the case of the fusion nucleus

respectively, the first bearing one of the two determiners for outward characters and the second one of the two determiners for seed-weight. Thus with the union of the proper tube nucleus and the fusion nucleus bearing the same "influence" the seed resulting from this fertilization would be heavy. With our present cytological knowledge of the processes involved, it is a matter of speculation whether this mature seed is heavy because of the relatively high specific gravity of the contents of the endosperm cells, or because the fully developed endosperm results from a large number of cell-divisions, or finally, because the endosperm is made up of a smaller number of very large cells (see East and Hayes, 1911, p. 101; Harris, 1911, and the literature cited therein). If, in the same fertilization we were describing above, the generative nucleus of the same pollen grain and the egg nucleus of the same ovule on uniting both carry the *macrophylla* influence, a heavy seed capable of producing a plant in F_2 which resembled *macrophylla* would be produced.

Other combinations of nuclei might give medium weight seed plants resembling either one parent or the other, or plants to express the composite appearance of which the terms "intermediate" and "blend" have above been used. Still other possible combinations might result in the formation of light seed in the mature ovary of an F_1 hybrid, a certain portion of which would give rise to plants in F_2 resembling one parent, another portion to plants resembling the other parent, and still another and larger portion to plants called "intermediates" and "blends."

Without attempting to include any schematic representation of the possible combinations of nuclei and the resulting weights of seed and F_2 generation plants, it will only be said that such a scheme seems to represent fairly closely the numerical proportions which we have noted above as occurring in the weights in the F_1 hybrid seed and in the appearance of the F_2 generation plants produced therefrom. Any such explanation of our experimental results is, as previously mentioned, only tentatively advanced and it is recognized that little can be claimed for it without more evidence than that which we are able to offer.

The question of Xenia and the double fertilization in the seed of maize (East and Hayes, p. 101, and the literature there cited), is the only matter which bears directly on the above explanation. The discussion given by Bateson (1909, p. 270), is interesting in this connection, yet we seem to have some experimental evidence which goes to show that the two nuclei brought into the embryo sac by the pollen tube do not bear similar or even corollated allelomorphs and that all the nuclei of the embryo sac are not similar in composition. The above explanation, in general, seems possible from a cytological point of view (Coulter, Barnes and Cowles, 1910, p. 269), and is as theoretically conceivable as certain other methods of interpretation which have recently been put forward.

PART II

QUANTITATIVE EXPRESSION OF IMPERFECT DOMINANCE IN THE COROLLA DIAMETERS OF THE FLOWERS ON THE HYBRIDS PRODUCED FROM THREE VARIETIES OF *NICOTIANA ACUMINATA* (Grah.) Hook.

CONTENTS

		PAGE
I.	Introduction	118
II.	Discussion of the experimental material	119
	(1) Description of experimental material	119
	(2) Basis of identification of our experimental material and description of a wild form of *N. acuminata*	119
	(3) Growing conditions—soil and climatic conditions	123
	(4) Description of the *Nicotiana* flower	125
III.	Technique and methods	126
	(1) Obtaining "pure" seed	126
	(2) Cross pollination	127
	(3) Cleaning of the seed	129
	(4) Sowing of the seed	131
IV.	Experimental work—1910	132
	(1) General habit of the parental types	132
	(2) Distinctions in corolla diameter and measurements of the same	133
	(3) Cross pollinations made between parents	135
V.	Experimental work—1911	136
	(1) Cleaning, sowing, and germinating of the 1910 seed	136
	(2) Number and condition of the plants in the field	137
	(3) General habit of the hybrid plants	137
	(4) Method of measuring corolla diameter	138
	(5) Results of the measurements	139
VI.	Summary of results	144
VII.	Discussion of results	145
VIII.	Literature cited	152
IX.	Explanation of plates	158

I. INTRODUCTION

Probably the most recent discussion of plant and animal breeding, especially from the point of view of the Mendelian discovery, contains the following statement in reference to dominance, that "unessential feature of the Mendelian phenomenon"—"The fact that the dominant and hybrid tall (in Mendel's classic experiment with *Pisum*) appear to us identical is probably no more than a measure of the crudeness of the means which hitherto have been adopted to distinguish between them—" (Darbishire, 1911, p. 38). Thus, leaving aside the question whether or not the condition and appearance of the hybrid in F_1 is an "unessential feature," the report on the quantitative estimation of the condition in which certain parental characters are inherited in F_1 seems of interest and value.

Despite such assurances as the above in reference to the rather unessential nature of dominance (Bateson, 1909, pp. 13 and 53; see, also, Stockberger, 1912, p. 152, and Baur, 1911, p. 52), a considerable number of references to the "Law of Dominance" are to be found in the literature on plant-breeding which has appeared since the rediscovery of Mendel's experiments (East, 1907, p. 40, and Brainerd, 1907). With either point of view, the F_1 generation, throughout the literature as it has been accessible to me, does not receive any great measure of critical attention. When dominance can be reported, the ground is cleared for a direct advance toward the hoped-for Mendelian segregation in F_2 and, on the other hand, when a strikingly intermediate hybrid appears in F_1 we look up the situation and find that we are justified in merely making a note of this relatively unimportant stage in our experiment and in reserving our efforts in anticipation of difficulties in properly interpreting the F_2 generation.

The present paper contains the results of a strictly quantitative investigation of an easily measured character—corolla diameter, in the hybrids of three varieties of *Nicotiana acuminata* which are to be distinguished from one another in respect to this character alone.

II. DISCUSSION OF EXPERIMENTAL MATERIAL

1. Description of Experimental Material

The experimental material is described in tabulated form as follows:

Variety number	I	II	III
Source	Cambridge Bot. Garden	La Mortola	Fort Bidwell, Cal.?
Identified as	*N. acuminata grandiflora*	*N. acuminata parviflora*	*N. acuminata* variety
Length of corolla tube	1½ to 1¾ cm.	1 to 1½ cm.	1 cm.
Color of corolla tube	white	white, purple at base	white
Diameter of corolla tube	3 to 4 mm.	2 to 3½ mm.	2 to 2½ mm.
Color of corolla limb	white	white	white
Shape of corolla limb	salverform	salverform	salverform
Diameter of fully extended flower	26 to 29 mm.	19 to 22 mm.	13 to 15 mm.
Radical leaves	acuminate, petioled	(as var. I)	(as var. I)
Cauline leaves	narrowly lanceolate and petioled	(as var. I)	(as var. I)
Height of mature plant	60 to 75 cm.	70 to 105 cm.	60 to 110 cm.
General habit	widely branching from the base	(as var. I)	(as var. I)
First grown in U. C. B. G.	1907	1908	1903 (?)
Plant number in the U. C. B. G.	$\frac{150}{07}$	$\frac{125}{08}$ and $\frac{192}{08}$	$\frac{53}{03}$

2. Basis of Identification of our Parental Material and Description of a Wild Form of *N. acuminata*

The identification of our experimental material as *N. acuminata* is based upon the description and figure given by Hooker (Bot. Mag. Tab. 2919; cf. also, Setchell, 1912, p. 24, plate 26).

This description seems to approximate variety I of our experimental material most closely. Comes (1899, p. 39) recognizes under *N. acuminata* "variety *grandiflora*" and "variety *parviflora.*" With the first of these we connect our large-flowered form (variety I) and with the second the form in which the corolla diameter is intermediate in size (variety II). Comes gives no definite measurements of flower size for varieties "*grandiflora*" and "*parviflora*" but the fact that he does recognize two cultivated varieties principally distinguished from one another in respect to corolla diameter makes it seem justifiable to identify the two cultivated varieties among our three parental types with those he names.

The small-flowered form (variety III) was first noticed in the University of California Botanical Garden some four or five years ago and it is possible that the original seed was sent from Fort Bidwell, California. At least, the small-flowered form was first noticed in a portion of the Botanical Garden in which the Fort Bidwell seed was originally sown (1903?). One *N. acuminata* plant came to maturity in that same part of the garden this past summer (1911), and a measurement of its flowers showed that a fairly small fluctuation in corolla diameter was operative in this type, which has been self-sowing itself for seven or eight years. Only eleven flowers were measured and their corolla diameters varied between 13 and 17 mm., with the average diameter approximately 15 mm. It seems probable that our small-flowered variety III is a particular form which has become segregated from among the plants which, in some generation previous to 1908, grew from the seed originally sent from Fort Bidwell. In 1908 the striking and uniformly small flowers borne by some one of the plants in the original garden plot attracted attention, and since that year variety III has been propagated by pure seed and has come true each year to uniformly small flowers on all its plants.

Plate 29 is drawn from fresh material of variety II of our parental types. Figure 1 shows a typical lateral from a vigorous plant with the narrowly lanceolate cauline leaves, secondary lateral shoots, a fully opened flower, a number of buds at the top and maturing seed capsules, all one-half natural size. Figure

2 and figure 3 are normal flowers of variety II and figures 4 and 5 are views of half mature seed capsules. Figure 6 shows a typical radical leaf, most of which by the end of the season are much torn or dried and obscured by the soil about the base of the main axis of the plant. The leaf shown in figure 7 normally occurs along the main axis up to one-third of the distance from the roots to the top of the plant and along the basal portions of the larger laterals.

Quite recently a considerable collection of wild plants, identified as *N. acuminata,* has been made in Niles, California. The plants were growing in, and on the sides of, a dry, sandy, river-bed in about equal numbers with *N. Bigelovii.* Approximately 75 measurements of corolla diameters of the flowers on these wild plants were made in the field. The fluctuations of corolla diameter in flowers on the same plant were usually less than 5 mm., but between different plants standing side by side the mean of the corolla diameters of their flowers varied over 6 mm. Thus one of the wild specimens bore flowers the corolla diameters of which varied between 12 mm. and 16 mm., with an average diameter of 13 mm., and on another plant in a slightly different situation the corolla diameters of the flowers varied between 15 mm. and 20 mm., with the average at approximately 19 mm. In other words, the wild varieties of *N. acuminata,* at least as we have found them in California, exhibit a wide range of fluctuation in corolla diameter of flowers on individual groups of plants and a relatively small degree of fluctuation in the size of flowers borne by the individual plants themselves. The largest flower found on the plants at Niles was one measuring 22 mm. in diameter and the size of flower most usually occurring, together with the general habit of the wild plants, duplicated rather closely the appearance of variety II of our experimental material.

Plate 30 was drawn from fresh material obtained in the above mentioned collection of the wild *N. acuminata* variety made at Niles during November, 1911. On comparing this plate with plate 29, in which variety II of our experimental material is shown, the resemblance between the wild and the cultivated varieties is evident. Figure 1 of plate 30 shows an entire young

plant drawn one-half natural size. Its cauline leaves correspond fairly exactly to those in figure 1 of plate 29, except that the waviness along the leaf margins of the leaves of variety II is not so strongly marked in the leaves of the wild variety. The third leaf from the base of the stem in figure 1 of plate 30 resembles figure 6 of plate 29, while figure 7 of plate 30 approximates the appearance of the leaf shown in figure 7 of plate 29, though in this last case, and as is very evident, the narrow leaf-tip of the wild variety does not arise so sharply from the broad cordate leaf-base as in the leaf of variety II. The corolla tubes of the flowers shown in plate 30 are both longer in proportion to the size of the flower and somewhat more dilated at the top than in the flowers of variety II. The calyx teeth also are longer in the flower and the calyx in the ripening ovary does not so completely envelop the maturing seed capsule as in the cultivated variety.

Varieties I and II have been grown in a number of Botanical Gardens under such names as *Nicotiana vincaeflora,, N. suaveolens,* as well as *N. acuminata.*

In the three varieties of *N. acuminata* described above we have individuals identical, within the limits of normal individual fluctuation, in almost every respect except the diameter of the flattened salverform corolla. This point of difference is plainly marked and entirely constant. On the first occasion, four years ago, upon which the three were assembled and grown near one another, Professor W. A. Setchell observed the sharp gradation in this character and the absence of other distinguishing and delimiting characteristics. During the past two years, in which this species has been turned over to me, a large number of measurements and careful examination confirm these observations. As has been said, the present paper deals with the general appearance and corolla diameter of the first hybrid generation resulting from crosses between these three varieties of *N. acuminata.* Seed is being gathered at the present time which will make it possible to continue the investigation along the same lines in the F_2 and, possibly, the F_3 generation.

3. Growing Conditions—Soil and Climatic Conditions

All three varieties have been cultivated continuously from the years named up to the present time in practically the same situation and under similar conditions. Constituting a part of a considerable collection of *Nicotiana* types that has been accumulated during the past ten years under Professor W. A. Setchell's direction and for experimental purposes, this species has received the special care universally accorded material propagated for research work. The only portion of the Botanical Garden at the present time available for experiments in plant-breeding—suitably fenced and near a small propagating house and cold frames—is not well adapted to the most successful pursuance of such experiments. The "inclosure" lies at the lowest level of any part of the garden, is not well drained and is much shaded at all hours of the day by eucalyptus trees which exhaust much of the vitality of the somewhat fertilized, natural adobe soil.

Climatic conditions in Berkeley during the out-of-door growing season—May to December—are very fairly constant throughout a given number of years. Extracts from the records kept at the University of California observatory follow:

	1909					1910					1911					
	August	September	October	November	December	August	September	October	November	December	August	September	October	November		
TEMPERATURE (Degrees, F.)																
Mean temperature	58.0	59.2	56.5	51.3	46.8	56.6	55.9	57.0	50.9	48.8	56.6	57.5	56.3		51.3	
Highest daily average	17th, 63.6	69.5	68.2	58.4	54.5	61.2	60.5	66.5	56.5	56.5	20th, 62.2	17th, 61.5	16th, 68.0	27th,	61.2	
Lowest daily average	30th, 54.6	54.2	49.0	42.2	40.0	53.2	52.8	51.9	43.2	42.2	6th, 54.0	20th, 54.5	29th, 52.2	11th,	41.7	
Maximum temperature	17th, 80.0	90.0	81.3	64.8	59.8	78.0	79.5	84.0	65.6	63.5	19th, 79.2	18th, 78.2	16th, 84.3	2nd,	71.0	
Minimum temperature	5th, 51.2	49.0	44.8	37.7	34.1	49.0	48.2	47.2	37.5	38.0	15th, 50.1	20th, 48.3	2nd, 46.0	11th,	36.0	
Monthly range	28.8	41.0	36.5	27.1	25.7	29.0	31.3	36.8	28.1	25.5	29.1	29.9	38.3		35.0	
Mean of daily maximum temperatures	69.2	71.0	64.6	57.9	52.7	65.0	64.8	66.6	57.9	56.0	65.3	65.6	66.8		62.0	
Mean of daily minimum temperatures	53.6	54.8	52.6	47.4	42.3	52.3	51.7	52.7	46.8	44.8	52.9	52.4	51.5		45.9	
Mean daily range	15.6	16.0	12.0	10.5	10.4	12.7	13.1	13.9	11.1	11.2	12.4	13.2	15.3		16.1	
Greatest daily range	25th, 26.7	32.7	22.8	16.6	16.2	26.5	29.0	25.3	16.5	17.7	19th, 27.3	3rd, 24.5	6th, 28.3	29th,	24.7	
Least daily range	30th, 9.0	5.5	4.0	3.8	4.1	7.2	3.8	3.4	4.3	4.7	4th, 5.6	1st, 7.5	28th, 4.6	9th,	10.2	
RAINFALL (Inches)																
Rainfall, dew and fog	0.00	0.78	1.34	3.43	7.24	0.00	0.06	0.60	0.87	1.80	0.00	Traces	.73		.46	
Rainfall, dew and fog, since June 30th	0.00	0.78	2.12	5.55	12.79	0.00	0.06	0.66	1.53	3.33	Traces	Traces	.73		1.19	
RELATIVE HUMIDITY (per cent)																
Mean relative humidity	88.0	85.5	87.6	89.9	85.0	88.0	89.0	84.6	91.0	88.0	89.6	87.2	85.6		81.8	
Maximum humidity	often, 94.0	97.0	97.0	97.0	97.0	97.0	94.0	94.0	97.0	97.0	often, 94.0	13th, 94.0	8th & 24th, 94.0	21st,	93.0	
Minimum humidity, 8 P.M.,	17th, 77.0	55.0	61.0	69.0	63.0	82.0	78.0	54.0	79.0	65.0	20th, 82.0	18th, 59.0	19th, 59.0	29th,	49.0	
Monthly range	17.0	42.0	36.0	28.0	34.0	15.0	16.0	40.0	18.0	32.0	12.0	35.0	35.0		44.0	
Greatest daily range	15th, 15.0	31.0	21.0	19.0	18.0	9.0	12.0	37.0	15.0	20.0	30th, 9.0	18th, 30.0	19th, 32.0		33.0	
Least daily range	often, 0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	often, 0.0	often, 0.0	4th & 21st, 0.0	13th, 16th,	0.0	
WEATHER																
Number of clear days	10	11	13	14	13	7	6	19	8	15	6	17	18		24	
Number of fair days	18	14	8	3	6	8	18	7	12	9	15	7	10		4	
Number of cloudy days	3	5	10	13	12	16	6	5	10	7	10	6	3		2	

The observatory stands within 200 yards of the Botanical Garden and on a portion of raised ground some 35 ft. higher than the garden.

4. Description of the *Nicotiana* Flower

In general the structure of the *Nicotiana* flower adapts it unusually well for hybridization experiments. In the case of *N. acuminata* the flower is amply formed—the corolla tube never less than 1 cm. in length, the diameter of the tube from 2–4 mm. and varying in diameter of flattened corolla limb from 13 to 29 mm. in the fully opened flower (see plate 29). It is vespertine in common with many species of *Nicotiana*, especially the white flowered forms. In the bud the limb of the corolla is somewhat plicate. The anthers—with the exception of the one standing lowest in the tube—are not shedding pollen until the corolla lobes are fully extended. The fifth anther stands 2–3 mm. lower in the tube than the others and is shedding pollen first and just as the flower opens fully. I have observed on many occasions that two or three hours may separate the two conditions and, in the case of a flower that opens fully and for the first time after dark, the anthers may not break until the following morning. The two-lobed stigma is receptive from six to eight hours before the flower in general is in anthesis and continues receptive for a considerable time after all the anthers are open. A castrated flower under the bag may be successfully pollinated toward the end of the bud stage or while the corolla lobes are still folded and during the middle of the day when the other flowers on the plant are completely closed and deflexed. There is no difficulty in self-fertilization naturally or under experimental conditions. It perhaps takes place naturally when the flower closes during the middle of the day. The corolla will withstand considerable mutilation and still persist otherwise normally. I have never observed that the buds are perforated or entered by insects nor that the plants in general were injured in any way by insect pests. It has been impossible to determine whether cross pollination is, under natural conditions, effected by outward agencies and, in the case of such a white vespertine species, this undoubtedly takes place at night, if at all.

III. TECHNIQUE AND METHODS

1. Obtaining "Pure" Seed

In obtaining "pure" seed—naturally self- (close) pollinated or artificially self- (close) pollinated seed from the protected flowers of a parent or a plant of the F_1 (or subsequent hybrid generations)—a vigorous lateral near the top of the main axis of the plant is selected and prepared by stripping off leaves, buds and small branches for a distance of a foot or more below the terminal buds and flowers. The choice of a lateral on the upper two-thirds of the main stem means nearly total absence of the seed which in the case of lower branches falls from above and adheres to the sticky, minutely glandular epidermis. All opened flowers and all but three or four buds are then picked off the terminal inflorescence with forceps and a fresh paraffin bag, placed over these remaining buds and secured at the base with a copper-wired pot label suitably marked, allows sufficient room for a number of flowers to expand normally and effectually excludes the entrance of foreign pollen by practically any agency. As mentioned above, self fertilization is accomplished readily and in the majority of cases, after the bag has been carefully opened at the end of two or three weeks to make sure that seed is being set, no further attention need be given the material until the seed is gathered at the end of another two or three weeks. In this connection it might be said that, in the case of *N. acuminata* the ripe seed often begins to fall from the promptly 2-cleft apex of the capsule while the lower two-thirds of the maturing ovary is still green. The semi-transparent paraffin bag makes it possible to watch the development of the capsule without opening the bag. If the seed capsule is gathered and hung to dry when the tip of the capsule first begins to show a brownish coloration, the seed ripens perfectly within the capsule and danger of a mouldy condition of the seed is eliminated.

In gathering seed the lateral is cut three inches below the bottom of the bag, placed and wired in a larger heavy manila bag with the pot label showing, and hung to dry out of doors.

2. Cross Pollination

In preparing flowers for cross fertilization a number of precautions and as exact a routine as the circumstances permit are rigidly enforced and any slip that can be detected serves to put a stop immediately to the work in hand. It can be fairly stated that the technique and methods developed in connection with all matter pertaining to the *Nicotiana*-experiments have reached a degree of refinement which cannot reasonably be surpassed without introducing minutiae of detail too complicated to be practicable. The small size and great number of seed borne by a *Nicotiana* plant introduce one of the greatest sources of error in these experiments and has led to unusual precautions, especially in the matter of cleaning, sowing and germinating the seed.

In the field the first precaution taken is one which undoubtedly is universally observed in such work (Shull, 1908a). The arms are bared to the elbows and arms and hands are sterilized in 95 per cent alcohol. A curve-pointed forceps and a small pair of scissors are kept immersed in 95 per cent alcohol when not immediately in use. Until the conclusion of the particular operation under way the hands touch only the instruments and the flower or flowers that are being worked with, and any contact between them and the clothing or other plants nearby necessitates at once a new sterilization. When an assistant is at hand it is entirely possible in making, for example, a cross-pollination, for the operator himself to do nothing more than pick out with the forceps a stamen from one flower which has been brought to him in its bag and apply the pollen-covered anther to the stigma of the female parent.

The male parent is prepared just as described above in connection with the obtaining of pure seed. The label is given the plant number with the male sign writen directly after it—e.g., $\frac{53}{03}$ ♂. In work on *N. acuminata* four or five sets of buds were prepared on a male parent at about the same time in order that there might be no doubt of a sufficient supply of pollen in case of any errors in the subsequent operations.

In the profusely branching species of *Nicotiana* much care is necessary in picking all buds out of the axils of leaves and thoroughly stripping the lateral chosen on the female parent so that small branches and flowers may not develop within the bag while the hybrid seed is being formed. The lateral is carefully gone over with the scissors and usually only one bud on the terminal inflorescences is left. The hands and instruments are now sterilized again. With the forceps the corolla tube is carefully split from the top of the calyx teeth to the corolla lobes, or until the corolla can be gently pushed back and the stamens and pistil exposed. The unopened stamens, one or two at a time, are then pinched off with the forceps half way down the filament, or often the anthers themselves are picked out separately. The corolla can now be closed back and around the pistil, the effort being to mutilate and derange the normal condition and position of the perianth as little as possible. A bag covers the castrated bud and the label attached to the bag carries the plant number and the female sign—e.g., $\frac{150}{07}$ ♀. It may be mentioned here that this label remains with the bag while on the plant, during the drying of the seed, and is placed in the seed envelope when the seed is cleaned. A number of such castrated buds are prepared and often the date and hour is noted on the labels attached. References to all such operations are, of course, placed in the record book for the particular experiment.

These flowers in both the parents will be ready for use in two or three days. When the two parents have been prepared at about the same time and the buds are of the same size I usually watch the pollen parent, since, as noted above, the stigma is practically sure to be receptive as soon as the anthers are open. This holds true for each of the three varieties of *N. acuminata* as well as for different flowers on the same plant. The developing buds can be seen through the paraffin bag and on the second day after bagging, when the corolla of the pollen parent is fully extended, it may be safely assumed that conditions are favorable in both parents for cross-pollination.

The lateral upon which the male parent has been bagged is

cut below the bag and brought unopened near enough to the female parent plant to be within easy reach. The copper wire with male label attached is unwound from the neck of the bag, placed upon the field table near by and the bag itself partially opened. The label from the female parent bag is next placed beside the male label and the bag loosened. When the hands have been sterilized the bag is lifted from the castrated flower and laid aside. The pollen parent is taken from its partly opened bag and a pollen-covered anther is extracted with the forceps and applied to the receptive stigma of the female parent. The proper bag is again placed over the pollinated flower and labeled with the original designation of the female plant with now in addition the name and sign of the male parent—e.g., $\frac{150}{07}$♀× $\frac{53}{03}$♂. If but one crossing operation at a time is attempted, and if the male and female labels are placed side by side during the short period when they are not attached to the plants themselves, there is little danger of mixing the parent labels with subsequent difficulties of identification.

3. Cleaning of the Seed

The seed is practically all gathered and dried before December 15, is cleaned during February and March and sown late in March and in April. The exceedingly small size and great number of seeds in a single capsule from a *Nicotiana* plant makes necessary the utmost precautions to prevent contamination in connection with the cleaning and sowing of the seed. During the past three years the seed has been cleaned in a different room on each occasion. One person is required to do the "bookkeeping" and wrapping of the seed envelopes and another to do the actual handling of the seed itself. The bookkeeper lays on a clean table a sheet (4 by 5 feet) of heavy brown manila paper and on it a smaller sheet (12 by 16 inches) of ordinary white paper. The seed cleaner then places a white earthenware saucer upon the white paper and receives the first bag of seed from the bookkeeper. The latter cuts off the tops of the bags, thus opening them and also freeing the seed label. He then

copies the hybrid or parent designation from the label onto two sizes of envelopes and returns to the "cleaner" with the smaller of the two envelopes. During the copying of the label, the cleaner has lifted the original paraffin bag from the larger manila bag in which it has been dried and has drawn out the withered stem and the seed capsule or capsules. Usually most of the seed is still retained within the capsule and the cleaner "shells" it out into the saucer and discards any seed which may have fallen out into the bag. The rougher chaff, etc., present may be picked out and laid on the larger brown paper.

The seed is then poured from the saucer onto the white paper and from it into the smaller of the two envelopes which the bookkeeper holds out. He seals the envelope, makes two folds over the sealed end, places the envelope and the original label on a small sheet of white paper and wraps them securely. This paper is then put into the larger envelope and the latter sealed. During this last operation the cleaner has laid aside the two sheets of paper, cleaned the saucer and thoroughly washed his hands and wrists. The bookkeeper now lays out two clean sheets of paper, the saucer is replaced and another bag of seed cleaned. The bookkeeper, of course, takes notes upon the yield and condition of the seed in each particular case. It will be noted that only one person actually touches the seed, that he has only this part to play in the whole operation, that his hands are thoroughly cleaned each time, and that every effort is made, in general and in particular, to insure cleanliness of surroundings for the work. The danger of mixing labels and making mistakes in copying is limited, since each package of seed contains three identifying marks. The careful sealing and wrapping of the seed envelope is necessary because of the small size of the seed and the fear of loss and contamination, should there be any possibility of its working out of the package.

4. Sowing of the Seed

The sowing of the seed is usually commenced during the latter part of March. Pots, 8 inches in diameter and 2½ inches deep, have been especially provided. They are filled with a mixture of sand and garden soil and sterilized in the autoclav for three hours, at eighteen to twenty pounds pressure. For each pot cheese-cloth covers suitably cut are sterilized at the same time. When the pots come from the autoclav these coverings go on at once and are secured by wire rings about the pots.

Two persons also are needed in sowing the seed. Two sizes of clean paper are again laid upon the table and the covered pot placed upon them. The bookkeeper gives the first package of clean seed to the "sower" and, lifting the covering off the pot, lays it on the white paper always with the side that has been nearest the soil uppermost. The sower then opens the outer seed envelope, unwraps the paper, and compares the label with the designations on the two envelopes. If no mistake has been made and the three agree, the sower reads the seed number from the label and it is checked off on the bookkeeper's list. The sower now tears open the seed envelope proper and shakes out the seed rather thickly over the surface of the soil. Any seed remaining is wrapped up again and laid aside. Granite pans, one for each pot and previously sterilized in the flame, are used to press down the seed into the earth. The bookkeeper now returns the cloth covering, secures it and writes the proper designation on the side of the pot. The two papers and the pan for pressing down the seed are now discarded, the sower washes his hands and the whole operation is repeated.

Since this paper constitutes one of the first of a considerable series on breeding investigations in *Nicotiana* which will probably be published from this laboratory, under Professor Setchell's direction, it has seemed necessary in the above to go with some detail into the technique he has adopted, which holds rigidly for this particular experiment and in general method for all other allied investigations in the Botanical Garden of the University of California.

IV. EXPERIMENTAL WORK—1910

1. General Habit of Parental Types

In 1910 the seed of *N. acuminata* was sown on March 14 and taken to the propagating house on the same day. The propagating house was thoroughly cleaned and sterilized and the ventilators covered with cheesecloth. The pots were watered from beneath, with the covers on, and these last were replaced in two or three days by glass plates. I have no record of the time of germination or of its amount. The glass plates were not lifted from the pots until the size of the young plants made it necessary. Quite generally toothpicks or pot labels were slipped under one side of the glass so that there might be a slight circulation of air within the pot. After the glasses were removed, much care was taken never to lift or pass one pot over another. When the cotyledons were large enough to be picked up and held between thumb and forefinger, twice the required number of plants was "pricked out" into flats. These wooden boxes or flats—18 by 18 inches—were filled with rich garden soil and the plants so spaced that each flat held 25 seedlings. In "pricking out" a fresh toothpick is used for each seedling to loosen the earth and scrape off soil particles which come up with the fine roots. By carefully spacing the plants in the flat and watching their development every day or two, there is little danger of any foreign seed germinating and getting established before being detected. When in the rosette stage and about the tenth of June, the required number of plants was lifted from the flat and placed in the portion of the inclosure set aside for them. The remaining plants in the flat were held for a month in case of accident to those permanently set out. Lack of room necessitated the growing of a relatively small number of the *N. acuminata* plants as well as undue crowding of these. When I saw them first in August there were six plants of variety I, eight of variety II, and fourteen of variety III. In each case the groups of plants were spaced two feet apart, the rows of plants one foot apart and twelve inches left between the plants in the row. Varieties II and III were within six feet of each other and variety I was some distance away.

On August 20, the three varieties were fully developed and covered with flowers. In general habit variety II and variety III were almost identical. Some trace of fasciation was observed in two plants of variety III and its habit throughout was a trifle the least robust. The leaf characters were the same in these two numbers, though variety III has always exhibited a somewhat ragged appearance in this connection which is not shared by variety II, but is also present, though less pronounced, in variety I. Variety I was growing in an especially unfavorable situation and seemed stunted and far from vigorous. One plant, however, stronger than the rest, compared very closely with the members of variety II and variety III.

2. Distinctions in Corolla Diameter and Measurements of the Same

The distinctions in corolla diameter were most clearly marked as shown in figure 2 on plate 34. This photograph was taken in 1908, but the gradations in size hold good equally well for the years 1910 and 1911. Some three hundred measurements of the corolla diameters of the three varieties were made during August and September, 1910. Corolla diameter is taken to mean the average of two measurements at different angles across the flattened surface of the fully opened salverform corolla limb —(plate 32, A to B and B to C). A flexible celluloid millimeter ruler was used and simply laid on the flower at the two angles and the measurements recorded. The condensed record of these measurements follows:

Variety number	Number of flowers meas.	Diameter of smallest flower	Diameter of largest flower	Average all measurements	Degree of fluctuation
I.	108	26 mm.	28 mm.	26.93 mm.	3 mm.*
II.	131	19 mm.	22 (23?) mm.	20.66 mm.	4 or 5 mm.*
III.	82	13 mm.	15 mm.	13.98 mm.	3 mm.*

(* The fluctuation between diameters 13 mm. and 15 mm. amounts, of course, to a difference of but 2 mm. By speaking of the fluctuation as amounting to 3 mm. is meant that corollas of flowers of variety III were measured which were found to be 13 mm., 14 mm. and 15 mm. in diameter. The same method of notation for matters of degrees of fluctuation holds throughout in the above table and in similar tables expressing fluctuation in parent and hybrid diameters).

In reference to the diameter of the largest flower measured in variety II there is doubt about extending the limit to 23 mm., since but one record of this diameter was noted and the figure "3" is somewhat obscure in the original record. It may be claimed that a degree of variation in corolla diameter as slight as that noted in variety III—13 mm. to 15 mm., or 3 mm.—or even in variety II of 5 mm., is impossible. A superficial glance at the plants in flower would certainly seem to substantiate such a claim. The diameters given, however, have reference to measurements made upon flowers of the same age or, better, in the same stage of development. Only such flowers were measured as exhibited all the anthers shedding fresh pollen. It is not a difficult matter to decide by a far from critical examination whether or not this condition is present in a given flower and, after a considerable acquaintance with the flowers of *N. acuminata*, it has been found possible to decide very quickly.

All the measurements in 1910 were made between 7:45 and 9:30 a.m. The newly opened flowers often show the corolla lobes fully extended and flattened but the anthers still green. There is a certain characteristically immature look about such a flower very easy to recognize at a glance. In such a flower on a bright, warm day the anther lowest in the tube will usually break during the afternoon and on the following morning all the remaining four will be shedding pollen. I have found that the difference in corolla diameter in these two conditions usually exceeds 2 mm. in all varieties of *N. acuminata*. Thus adding 2 mm. to the corolla diameters for the two extremes given for variety I we might have on the same plant flowers measuring 24 mm. and 30 mm. and all sizes intermediate. Likewise for variety II, 17 and 24 mm. and for variety III, diameters measuring 11 and 17 mm. and all diameters between. If we add, again, the appearance of partially opened buds and flowers past anthesis, there would seem to be on a plant, for example of variety II, the greatest individual fluctuation in corolla diameter. Still it may be seen that, allowing 2 mm. beyond either extreme of fluctuation in corolla diameter of fully opened flowers as noted in the above table, there is yet a definite division into the three varieties—i.e., a large (24 to 30 mm.), a small (11 to 17 mm.),

and an intermediate variety (17 to 24 mm.) This matter will be further discussed in connection with the measurements of corolla diameters of hybrids and parents in 1911.

3. Cross Pollinations made between Parents

Between September 2 and October 5 seventeen cross-pollinations were, to all appearances, successfully performed. The cross I ♀× II ♂ was made twice and its reciprocal II ♀× I ♂ twice. Variety I was crossed with variety III on two occasions and III ♀× I ♂ twice also. Cross II ♀× III ♂ was made five times and III ♀× II ♂ four times. To obtain pollen for these cross-pollinations twenty-two sets of buds were prepared and bagged and twenty-eight female parents were castrated and bagged. In variety III there was a most annoying tendency of the buds that were being worked with to snap off and it was quite difficult to perform the castrations successfully. Fully opened flowers were not so delicate in this respect. Fourteen of these crosses had been made before September 7 and on the 20th the bags were opened and a few small buds, inconspicuous and overlooked in preparing the lateral two weeks before, were picked off. In all but three cases seed seemed to be forming. The three doubtfully successful crosses were repeated. All seed was gathered and hung to dry before October 15.

Because of the extent to which variety III dropped its buds this parent was artificially self- (close) pollinated. In the other two varieties there was no difficulty in obtaining naturally self- (close) pollinated seed. The parental seed was gathered and hung to dry along with the hybrid seed.

V. EXPERIMENTAL WORK 1911

1. Cleaning, Sowing and Germinating of 1910 Seed

The *N. acuminata* seed was cleaned in April, 1911, and sown early in May. As it was not possible for me to observe the material before August, the sowing was done later in the spring than is usual. The germination and entire direction of the planting out, etc., was in the hands of Mr. W. G. Perrine, the head gardener of the Botanical Garden of the University of California, of whose unfailing interest and enthusiasm, ability and absolute integrity, acknowledgement should here be made. At the time of cleaning the seed it was found that there would be sufficient hybrid seed for sowing but none to carry over to be grown with the F_2 generation. Among the parents it was found necessary, in order to obtain sufficient seed of variety III, to use the remainder of the seed sown in 1910. The scarcity of hybrid seed was due only to the fact that I had not thought it necessary to repeat the various crosses more than once and not to any lack of seed in the two capsules which in each case resulted from the cross-fertilization. The cross II ♀× III ♂ and III ♀× II ♂ yielded four capsules of seed from the nine attempted cross-pollinations—two from each cross. The cross II ♀× I ♂ unfortunately gave no seed in either of the two attempts above mentioned.

The germination of both parent and hybrid seed was high. Twenty-five seedlings of each of the parents and of all the hybrids except cross II ♀× III ♂ and its reciprocal, were pricked out into flats. In this last instance the seed from each of the four capsules was cleaned separately, germinated in the individual pots and carried into the field with distinguishing labels.

The small plants were set out late in June and occupied one whole side of the inclosure. In this case there was sufficient room available so that there could be four feet between groups of plants, two feet between rows and twelve inches between plants in the row. The months of June and July were unusually cloudy and cold and the development of the plants in the field was correspondingly slow.

2. Number and Condition of the Plants in the Field

It was not found possible to work with the *N. acuminata* plants in the field before the first week of August. Among parents and hybrids the following table gives the number of vigorous plants in each case at this time, the dates upon which each group first flowered, the date upon which the last flowers were measured and the number of plants left on November 20.

Parent and hybrid number	Number of plants Aug. 10	First flowers measured on	Last flower measured on	Number of plants on Nov. 20
Var. I	5	9/3	10/4	3
Var. II	15	8/31	11/6	7
Var. III	10	9/7	11/6	3
I ♀× III ♂	7	9/8	11/20	2
III ♀× I ♂	19	9/6	11/6	15
II ♀× III ♂	36	9/5	11/20	18
III ♀× II ♂	29	9/9	11/18	20
I ♀× II ♂	12	9/8	11/20	10

The parents, though on the same strip of land with the hybrids, were far from being vigorous plants and remained throughout, with the exception of variety I, stunted and sickly. In variety I the plants were identical with the smaller individuals described in the previous year's work on this number. The flowers were, of course, in all the parents not nearly so abundant as in the previous year and in the case of variety II it was possible to measure only ninety-nine flowers. The leaf and general habit characters were again identical, though, as noted, the plants throughout all three varieties were smaller in vegetative characteristics than as observed in 1910.

3. General Habit of the Hybrid Plants

Among the hybrid plants each plant has been carefully gone over during the season and the following points noted. The leaves, cauline and radical, were exactly the same throughout. It has not been possible comparing leaves from corresponding regions on any two plants to tell them apart except in the matter of slight variations in size, nor are there distinguishing marks when compared with the leaves of 1910 and 1911 parents. In habit the hybrids were identical with the habit of the parents

in 1910 and also among themselves except as follows. One plant resulting from the cross I ♀× III ♂ was in habit exactly as all the plants of variety I in 1911 and as all but one in 1910. Three plants of I ♀× II ♂ were not up to the normal in height and were more broadly spreading, with thicker main stems and laterals. Lastly, five plants of III ♀× II ♂ were very small and in appearance immature, with few small laterals and small leaves. The traces of fasciation observed in two plants of variety III in 1910 appeared in five plants in 1911 but only in those hybrids which had variety III as a male or female parent.

4. Method of Measuring Corolla Diameter

Measurements of corolla diameter of the parent and hybrid flowers, were first made on September 8 and the last record noted is on November 20. Slightly over 2750 measurements were recorded during this period. A sheet of zinc, 3 by 4 inches, was deeply notched and served as a very simple holder for the flower while being measured. If the flower is picked off, gently slipped into the notch and pulled down until the flattened corolla lies upon the surface of the zinc plate, the ruler can be laid firmly on the face of the corolla, at the desired angles, and the two measurements taken very readily and after some experience, sufficiently exactly. Other devices such as a squared sheet of smooth paper ruled in millimeters, upon which the flower could be pressed and the diameters read off, were found to be less accurate than the more simple apparatus described above.

On an average two or three flowers can be accurately measured a minute. Considerable delay was caused by the necessity of frequently washing off in alcohol the heavy gum from the stems of the tobacco plants, which covered the hands and instruments to such an extent as, at times, to render further work impossible. An assistant recorded the measurements, thus simplifying the work greatly. The records were so taken that it is possible to refer, on a given date, to the measurements of flowers on any particular plant in the whole series and, in a general way, to recognize the region on the plant upon which any single flower occurred. All measurements were made between 8 and 10 a.m. with the exception of November 9 and 13,

upon which dates the flowers were measured between 5 and 6 p.m. The greatest effort was made to exclude personal bias in connection with the measurements. As the plants were worked with nearly every other day for over three months it was impossible not to form a more or less definite idea of the direction towards which the results were tending. Also, when measuring fifty to sixty flowers on one day from a single group of plants, one could scarcely avoid keeping certain limits of fluctuation in mind. It was attempted, however, to make the measuring as mechanical an effort as possible and the actual limits of fluctuation, as given below, were not found until practically the last record was taken. The averages of the measurements, also, were not calculated until all the measurements were completed and the experimental portion of the work concluded.

5. Results of the Measurements

The following table gives the number of days upon which the flowers in each group were measured, the largest and smallest number of flowers measured on a single day, the total number of flowers measured, the largest and smallest diameters and the average diameter in each case, the theoretical size of the true intermediates between the average diameters of the parents, and the fluctuations in corolla diameter throughout.

Group number	Var. I	II	III	III♀× I♂	I♀× III♂	I♀× II♂	II♀×III♂	III♀× II♂
Number of days upon which plants were measured	26	11	24	31	15	27	24	23
Largest number measured on a single day	18	15	17	86	9	17	33	65
Smallest number measured on a single day	1	1	1	13	2	3	3	7
Total number measured	228	99	185	849	36	320	374	757
Diameter of largest flower, mm.	29	22	15	27	23	30	20	22
Diameter of smallest flower, mm.	26	19	13	17	17	23	13	14
Average diameter, mm.	26.9	19.8	13.6	21.63	19.69	24	16.94	16.8
Intermediates between averages of parents, mm.	.....	.....	.....	20.25	20.25	23.35	16.7	16.7
Fluctuation in corolla diameter, mm.	4	4	3	11	7	8	8	9

Here again we have a very small degree of variation in the corolla diameters of the parents. The measurements were very carefully taken and at times a few measurements would be made upon the parents, then upon the hybrid plants, and again upon the parents. It was possible in 1911 to watch the plants as they first came into flower and every effort was made to follow the development, from the bud to the fully opened flower, of every individual flower measured either on hybrid or parent plants. By this is meant that, for example, on September 28 it was observed, in the case of plant 1 of the cross III ♀× II ♂, that two flowers were in a proper condition (see page 134) and they were picked off and measured. On the same date and on this plant three flowers were almost in the proper condition to be measured—i.e., the anther lowest in the tube shedding pollen and the corolla fully extended but the remaining anthers unopened. There were also four flowers all with the anthers green and the corolla only partially opened as well as a considerable number of rather small buds. Now in this case, it was plain that the anthers of the first three flowers mentioned above would be shedding pollen during the afternoon of September 29, and that on the following morning these two flowers would be in the proper condition to be measured. It was also evident that some of the remaining four fully formed buds might be properly developed by that time. At eight a.m., September 30, only three flowers could be measured. One of the three flowers almost ready the morning before had evidently begun to shed pollen from all the anthers before noon, for in this flower two of the anther cells were almost free from pollen, the brownish coloration at the back of the pollen receptacles was showing, and the stigma was covered with pollen. In the other two the anthers were balls of light, fluffy pollen and no pollen could be noticed on the stigma. All three of these flowers probably could rightly have been included in the measurements so far as development in corolla diameter is concerned, but only the last two were considered to be in exactly the proper condition and the third was picked off and thrown away. Of the four flowers that had been quite undeveloped on September 29, two showed the anthers lowest in the tube shedding pollen, the third flower showed all the anthers

still green and the fourth flower only was in the proper condition to be measured. On September 30 there were, in addition, four newly opened buds. The next measurements on this plant are dated October 2, on which date four flowers were measured—i.e., three of the four that were newly opened buds on the 30th and one which had come to maturity since that time.

In other words, when the measurements were finished on each day, only small buds and immature flowers were left on the plants. By taking the measurements every other day there was usually an abundance of material and most of it in the same condition. The foregoing applies, of course, to measurements on the parent as well as on the hybrid flowers.

Plate 31 gives the number of flowers measured on sixteen or seventeen different days, between October 1 and November 8, on single plants of the various groups and, in the case of the plotted figures in the upper right hand corner, the number of flowers measured on all the plants of the group representing the cross I ♀× II ♂. The plant upon which the greatest number of flowers was measured during the five weeks is, in each case, given. The close similarity between the plotted figures is rather striking. Climatic conditions (see page 124), operating on all the plants, undoubtedly is the chief cause of this similarity, though a probable periodicity in the production of reproductive parts, as opposed to those strictly vegetative, may enter in as a factor, It hardly seems, however, that this last can have great significance in reference to the similarity between the plotted figures, since all the plants would not naturally be influenced by such a periodicity on practically the same dates. Between October 20 and 30, when the production of flowers was in general exceedingly low, there had been rain, cloudy weather and an extremely low temperature at night, following a period of climatic conditions practically the reverse. Probably the direct result, in this connection, was seen in a tendency throughout to drop the flower buds. More favorable weather conditions—November 1 to November 8—always caused an immediate and rather disproportionately large increase in the production of flowers.

The degree of variation in corolla diameter among the hybrid plants is, in each case, large. For example, III ♀× I ♂ varies

between 27 mm. and 17 mm. and III ♀× II ♂ between 22 and 14 mm. The relative degrees of fluctuation in corolla diameter are well brought out in plate 32. All the drawings with the exception of that marked II♀× III♂ were made from fresh material, suitably selected to show the extremes of variation among parent and hybrid plants. It must be emphasized that these extremes of variation were continually observed on single plants of the various groups of hybrids and did not occur simply as isolated instances in the measurements of an entire group. Thus, corolla diameters in plant 2 of III ♀× I ♂ vary as follows between October 2 and November 20. The daily average is also given.

Date 1911	Oct. 2	4	6	8	9	11	13	15	18
Largest flower in mm.	26	25	27	26	25	26	26	24	26
Smallest flower in mm.	21	20	23	21	22	21	21	21	17
Daily average in mm.	24	23.8	24.8	23.7	23.6	23.2	23.9	22	24.2

Date 1911	Oct. 20	27	30	Nov. 1	3	6	8	18	20
Largest flower in mm.	27	25	25	26	25	25	25	23	22
Smallest flower in mm.	19	20	19	19	17	18	18	19	19
Daily average in mm.	22.9	23.8	23.6	19.7	21.7	22.8	23.2	21.6	20.8

Thus on a single plant of the hybrid III ♀× I ♂ we obtain the extremes of fluctuation—17 mm. and 27 mm.—which the flowers of the entire group of plants representing this cross displayed. As has been said above, this was true throughout all the hybrid groups—i.e., practically *every plant* of a hybrid group exhibited in its flowers the extremes of variation in corolla diameter reported for the *whole* hybrid group. It might have been possible that toward the end of the flowering season the fluctuation in corolla diameter would be diminished or that the full effect of the amount of acquired characters might exhibit itself (Lang, 1908; see also Groth, 1911, p. 8; Moore, 1910, 1912). As will be seen from the above table and plate 33, the

degree of fluctuation was practically as great at the beginning, at the middle and at the end of the flowering season.

Plate 33 exhibits the degree of fluctuation in corolla diameter of varieties I and III and the fluctuation of all the plants representing the cross III♀× I♂ of each of sixteen different days between September 15 and November 6. The heights of the various columns correspond to the number of flowers for each diameter measured on the various dates. It will be remembered that the parental varieties did not give as vigorous plants during the season of 1911 as in 1910 and did not flower anywhere nearly so profusely as during the previous season. This is the cause of the shortness of the columns throughout for the parental varieties I and III as contrasted with the heights of the columns showing the fluctuation of the hybrid plants representing the cross between these two parents. A comparison between plate 31 and plate 33 will show that, in general, the production of flowers for the entire group of plants of cross III ♀× I ♂ (plate 33) corresponds closely to the production of flowers on a single plant of this hybrid group (plate 31).

It will be noted that upon only two occasions was the diameter 29 mm. found for variety I, while the limits of fluctuation of the hybrid, 17 and 27 mm., appeared on six occasions for the diameter 17 mm. and on seven different days for the diameter 27 mm. Diameter 15 mm. was noted on the flowers of variety III on only two occasions, both previous to September 15, and for this reason diameter 15 mm. is not included in the columns of variety III. It is also plain that the number of flowers in the hybrid group exhibiting these extremes of fluctuation was large as compared with the number of flowers giving the limit of fluctuation 29 mm. in the parent variety I. Also, from plate 33 and the above table (p. 142), it can be seen that there was no appreciable difference in the average size of corolla diameters as the growing season advanced; a difference which might have been expected to exhibit itself because of an increase of strictly vegetative growth at certain times and a consequent inhibition of normal floral development.

VI. SUMMARY OF RESULTS

The foregoing description of experimental results can be summed up as follows:

1. The experimental material consisted of three varieties of *Nicotiana acuminata*. The three varieties are distinguished from one another almost solely in the diameter of the limb of the salverform corolla of the flowers.

2. The differences in corolla diameter among the three varieties were practically constant throughout two seasons during which the corolla diameters were measured in the Botanical Garden of the University of California. The three diameters were 13 mm., 20 mm. and 27 mm., with fluctuations never exceeding 2 mm. either greater or smaller than the mean diameter in each case. The three mean diameters and the fluctuation noted were obtained on the basis of approximately 800 measurements of flowers of the three varieties.

3. From six successful cross-pollinations—the three crosses between the three varieties and the reciprocal crosses—five groups of hybrid plants were brought to maturity (100 plants at the opening of the season and 65 at the close). Approximately 2750 measurements of the corolla diameters of flowers on hybrid plants were made.

4. The measurements of the corolla diameters of the flowers on the plants of each group of hybrids gave an average diameter for each group which was practically the same as the calculated average between the corolla diameters of the corresponding two parents. In other words, each of the five average hybrid corolla diameters formed intermediates in size between the corolla diameters of the parents of the corresponding cross. Each cross and its reciprocal gave practically the same result in this connection.

5. Variety II, called an "intermediate," in reference to corolla diameter, is truly an intermediate between the large and small flowered varieties of *N. acuminata*. This is shown by the fact that when the large and small varieties are crossed the average corolla diameter of the hybrid flowers approximates

fairly closely the average corolla diameter of this "intermediate" variety.

6. Among the corolla diameters of the flowers of all the hybrid plants a wide range of fluctuation was observed. This fluctuation included diameters from 13 mm. to 30 mm.

7. The fluctuation 13 mm. to 30 mm. is as great as the difference between the smallest corolla diameter of variety III (the small-flowered parental variety)—i.e., 13 mm.—and the largest corolla diameter of variety I (the large-flowered parental variety)—i.e., 29 mm.

8. The maximum fluctuation in corolla diameter of a single hybrid group of plants was 11 mm., or almost three times as great as the parental type which fluctuated most widely—i.e., 4 mm.

9. The minimum fluctuation for a single group of hybrid plants was 7 mm., or almost twice as great as the largest fluctuation in corolla diameters of the flowers of the three parental varieties.

DISCUSSION OF RESULTS

One important exception must be noted to the statement made in the introductory paragraphs of this paper, to the effect that no critical attention has been given to F_1 generation hybrids. Reference is made to Darbishire's work on the nature of the starch grains of round and wrinkled peas (Darbishire, 1908). As will be remembered, the cross between a round pea with potato-shaped or *p* starch grains and the wrinkled pea with compound or *c* starch grains yields a hybrid starch grain in F_1 which is intermediate in shape (length-breadth index), intermediate in the distribution of compoundness, intermediate in the degree of compoundness and the hybrid round pea itself is intermediate in absorbtive capacity between the parents. In F_2 "the homozygote round peas contain *p*-grains, the heterozygote round peas contain *r*-or intermediate, grains." Since this work of Darbishire's is practically the only experimental evidence so far accumulated which is comparable with that which has been reported in the present investigation, it is necessary

to determine the extent to which the experimental results agree. In the summary of results given on page 144 the statement is made that the average hybrid corolla diameter is intermediate in size between the parental diameters (see East, 1912, p. 247; also Hayes, 1912). To this extent our results *seem* to coincide with those reported by Darbishire. The difficulty is that, as shown in the figure on page 125 (Darbishire, 1908), there are no hybrid starch grains as round or as compound as the parental *c*-grains, nor are there any hybrid grains as potato-shaped as the parental *p*-grains. The hybrid grains are half-way between being potato-shaped and being round and half-way between compound and not at all compound—that is, they are true intermediates. The corolla diameters of hybrid flowers are, however, both as large as the diameter of the large-flowered parent and as small as the diameter of the small-flowered parent; both extremes occur among the flowers of a single plant; and, finally, only a numerical average establishes an intermediateness of corolla diameter for the flowers of a hybrid plant. The important point also is that, breeding from a hybrid pea the starch grains of which exhibit one-half the *D* influence and one-half the *R* influence, we might be prepared to have the hybrids in succeeding generations exhibit some definite degree of segregation. Similarly, if the hybrid corolla diameter was truly intermediate, in that it exhibited as above one-half the *D* influence and one-half the *R* influence, we might predict segregation in F_2 at least to the extent to which it took place in the starch grains of the pea hybrids. But now, when on a single plant of the *Nicotiana acuminata* hybrids flowers appear the corolla diameters of which show the unimpaired *D* influence and the unaffected *R* influence and every degree of intermediateness between the two, what prediction can be made as to the appearance of the F_2 individuals with respect to the corolla diameters of their flowers? In other words, is there evidence to show that largeness of flower vs. smallness of flower, intermediate flower size vs. small flower size, large flower size vs. intermediate flower size, etc., in each case, constitute the two members of a Mendelian pair?

It is interesting in this connection to note the partial report of an experiment in which peas differing widely in size were

crossed (Darbishire, 1911, p. 241). The F_1 hybrid was a blend, corresponding, probably, to the intermediate position occupied by the F_1 hybrid starch grains above mentioned, and, "so far as data at present available show, segregation does not occur when the hybrids are self-fertilized." The author raises the question whether, in such experiments, the F_2 generation might not consist "of a complete series of gradations between a 'small' identical with the pure 'small' at one end, and a 'large' identical with a pure 'large' at the other end" (Darbishire, 1911, p. 241; but see also Castle, 1905, and Bateson, 1909, p. 251). The possibility at once suggests itself that just this same condition of partial segregation is present in the F_1 hybrids of the *N. acuminata* crosses—in other words that, just as large seeds, small seeds and seeds showing all gradations in size between large and small might occur in the ripe pods of the hybrid peas in F_2, so on a single plant of the *N. acuminata* hybrids in F_1 large flowers, small flowers and all degrees of intermediate flower size occur. As the author suggests, again, the only practical value of continuing to breed from material showing such a type of inheritance is the possibility that one or both the extremes of the series may breed true in later generations and that a certain proportion of the intermediate forms, probably those nearer the extremes, may also breed true. With this in mind, it has been possible to secure F_2 seed from hybrid flowers on one plant, the corolla diameters of which were large, small, and intermediate in size between large and small. Thus the suggestion that can in general be made is that such segregation as will definitely occur (Bateson, 1909, p. 241) in respect to the corolla diameters of hybrid flowers of *N. acuminata,* is exhibited in the flowers of the *F_1 generation hybrids* and that the F_2 generation will show whether or not certain corolla diameters breed true or whether the fluctuation in corolla diameters which appeared in the hybrid flowers will be regularly diminished in F_2 and succeeding generations until the two parental types are ultimately regained and a number of new "flower-size" varieties, with small fluctuations in corolla diameters, are established.

Certain results obtained by Groth (1911, part 1, pp. 5–10) in his examination of the cotyledons on F_1 hybrid tomato seed-

lings are also interesting in this connection (see also, Gregory, 1909, and Gard, 1911). The fluctuation in the length of the parent cotyledons was great in each cross, — never less than 5 mm. and usually greater than 10 mm. — and the degree of fluctuation of cotyledon length in the F_1 hybrids, considerably diminished (*loc. cit.*, p. 7), for many of the crosses. A rather strange predominance throughout of the female influence in each cross is reported (see Hagedoorn, 1908; Loeb, King and Moore, 1910). The general result of the experiments seems to show that the "absolute size . . . . tends to be larger in the cross than the mean between the corresponding characters of the parents" (Groth, *loc. cit.*, p. 33). The fluctuation in cotyledon length almost always equalled and surpassed the higher extreme, but in no case reached the lower extreme of the parental fluctuations. It is in this connection especially that Groth's results differ from those reported in this paper. In the case of tomato seedlings it might possibly be held, since the *D* influence was practically always strongly evident; since the *R* influence was likewise absent, and finally since the average length of the hybrid cotyledons was nearly always greater than the mean between the average length of the parent cotyledons, that in respect to length of cotyledons, "longness" is dominant over both "shortness" and "intermediateness." It will again be repeated, for comparison, that in reference to corolla diameter in *N. acuminata* hybrids both *D* and *R* influences were always present, and the mean between the average corolla diameters of the parents was practically identical, in every cross, with the average corolla diameter of the hybrid in each case. In a second report upon the mature F_1 tomato hybrids Groth finds that in the seedlings "opposing factors . . . . are still struggling for mastery . . . . an equilibrium is reached when the plant becomes older, and that equilibrium is generally the same for the same combination of factors." This equilibrium is often shifted, however, "so that even in grown plants reciprocal and duplicate crosses may differ in characters of size, shape and number" (1911, part 2, p. 11). The fact that the F_1 generation of *N. acuminata* hybrids is far more variable in respect to floral

diameters than the parents must again be noted in connection with a recent report by Hayes (1912) and the discussion therein given and the previous observations by Johannsen (1906).

The use of the term "fluctuation" throughout this paper has been somewhat loose (Bateson, 1909, p. 240; cf. also, East, 1910, p. 82). In reference to the variations in corolla diameter exhibited by the parental varieties of *N. acuminata,* the use of the word "fluctuation" for such variation is undoubtedly valid. In using the same term for the variations in corolla diameter of the hybrids, a matter of convenience rather than strict definition has been consulted. It is probable that such a degree of variation in a definite floral "unit character" as that shown by the fluctuation in corolla diameter of the *N. acuminata* hybrids in F_1, has never been reported. That such great variation is due to "disturbing effects . . . . of environmental origin" (Bateson, 1909, p. 239; see also Hayes, 1912) is doubtful, and it is hard to see where the results arrived at in connection with pure-line breeding are applicable (Johannsen, 1909). It is moreover clear that the increased vigor generally assigned to the vegetative and floral characters of hybrids (Groth, 1911, part I; East, 1909, pp. 174 and 177) is not the force compelling the great variation here, for neither was the habit and foliage of the hybrid of a more luxuriant type nor (and this is the important point) did the hybrid corolla diameter in any case exceed by over one millimeter the diameter of the large-flowered parent in any cross.

The last point we must take up refers to the purity of the experimental parental material. To the best of my knowledge, the three parental varieties of *N. acuminata* have come true to certain definite corolla diameters for at least four years. Certainly during the past two years direct measurements have shown that the three varieties are definitely distinguished from one another by certain sizes of flowers, the fluctuations in the corolla diameters of which are so small as to cause no intergrading of one variety into another. The probable existence of the small-flowered variety as a wild type before its introduction into the Botanical Garden eight years ago, may suggest

that the particular type which we have considered to be the small-flowered *N. acuminata* variety (III), was of recent hybrid origin. As has been seen, nothing in the appearance in F_1 of any of the crosses which contain this small-flowered variety as a parent suggests that we are dealing with a heterozygote in the case of variety III. Certainly in the result of the crosses where the parents with large and intermediate flowers are concerned, there can be practically no doubt as to the homozygous condition of the parental material; and the appearance of the hybrid between these varieties was practically the same as the appearance of the hybrids which resulted from crosses in which the small-flowered parent was concerned. The number of plants brought to maturity in both the experiments reported upon in this combined paper is small but compares favorably with the numbers raised in experiments to the results of which a strict Mendelian interpretation has been applied (Price and Drinkard, 1908). It is to be noted, however, that the flowers upon only two generations of the parent plants have been measured and that thus we may not have fulfilled the criterion set by Johannsen when, speaking of "the hybridisation of types that are quantitatively characterized," he justly states that they "must be pure lines, the constancy (or, if it may be, the mutability, segregative capacity, and so on) of which has been previously studied in a sufficient number of generations" (Johannsen, 1906, p. 105). To sum up the above discussion, it may, in general, be said that the occurrence of flowers on *any one hybrid plant in* F_1, the corolla diameters of which show the unaffected "dominant" influence, the unaffected "recessive" influence, and all diameters intermediate between the corolla diameters of the parents, makes it difficult to find an explanation for such a type of inheritance according to any of the most widely accepted hypotheses. The discussion of "intermediates" (see Macfarlane, 1895) as given by Bateson (1909, pp. 235–241) and the four important classes within which intermediate types fall, does not seem to offer the necessary explanation. The "presence" of both the unimpaired *D* and *R* influences in F_1 makes it rather difficult to apply the "presence-and-absence hypotheses" (Shull, 1909) to a discussion of the experimental results herein reported. Again, none of

the parental corolla diameter characters are "latent" (Shull, 1908, p. 7 and the literature there cited) in F_1 but appear both fully activated and in various degrees of blending and combination. Finally, there is no evidence which goes to show that there is a "variability . . . . in the potency of determiners" (Davenport, 1910, p. 135) in our experimental material or that we are here dealing with the case of a "stronger determiner meeting a weaker determiner in the germ" (Davenport, 1908). It must at times be somewhat a matter of speculation just "what does meet what" in the germ and, in the case with which we are dealing, it seems more than commonly a subject for speculation.

We can, in conclusion, simply suggest that the occurrence of large flowers, small flowers and flowers the corolla diameters of which show every degree of gradation between large and small in F_1, of a cross between a large-flowered and small-flowered variety of *N. acuminata,* represents the maximum degree of "segregation" which occurs in such a hybridization (see, in this connection: Locke, 1906, and Emerson, 1910). The F_2 plants grown from the seed produced by self-fertilizing the flowers of this hybrid may give us a few plants bearing flowers, the corolla diameters of which show small variation in size. Succeeding generations may reduce the variation still further until we have regained either one or both of the parental varieties which entered into the production of the F_1 generation, or have established a new "flower-size" variety which will thereafter come true to a certain corolla diameter of flower which varies only slightly in size.

The two sets of experimental results reported in this combined paper are, in a broad sense, identical. In one case it has been possible to carry through the evidences of segregation which appear in F_1 plants and in the seed which they produce, into the F_2 generation; in the other it has not as yet been possible to do so. In both cases evidence of segregation appears after the seed produced by cross-fertilization had been grown and before the seed that was to produce the F_2 individuals had been germinated. In both cases we have been forced to suggest explanations for the phenomena we recognize which are not strictly Mendelian. In one case the non-appearance of any Mendelian

ratio may be due to the small number of plants grown in F_2, while in the other case we have difficulty in interpreting the wide variations in the outward evidences of such segregation as was observed to occur in F_1, in terms of any Mendelian scheme of analysis.

In general, both experiments are concerned with the "question of the inheritance of bulk" (Darbishire, 1911, p. 241) in reference to which, as this same writer states, "the present available information is very scanty." We are thus justified in not endeavoring to formulate definitely any general hypotheses on the basis of our experimental evidence, but have attempted to suggest, merely tentatively, certain points of view which seem applicable to the problems in hand.

It is a pleasure to acknowledge my indebtedness to Prof. W. A. Setchell, at whose suggestion the experiments were undertaken and under whose direction they have been carried on, for much helpful advice and criticism; to the Department of Physiology of the University of California for placing at my disposal the use of the mechanical balances used in weighing the hybrid tobacco seed; to Mr. Sturla Einarsson of the Department of Astronomy for providing me with the Monthly Meteorological Reports of the Students' Observatory which are combined to form the table on page 124; and to the members of my family for much assistance in connection with the measurements of the corolla diameters of hybrid tobacco flowers and in preparing the manuscript for the press.

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EXPLANATION OF PLATES

PLATE 29

Nicotiana acuminata variety II.

All drawings made from fresh material.

Fig. 1. A typical lateral from a vigorous plant. ×½.

Fig. 2. A typical flower, entire. Natural size.

Fig. 3. The same, split longitudinally; the ovary intact. Natural size.

Figs. 4 and 5. The half mature seed capsule. Fig. 4, natural size; fig. 5, ×2.

Fig. 6. Typical radical leaf. ×½.

Fig. 7. Typical leaf occurring one-third the distance up the main axis. Natural size.

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1
6
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4
5
7

PLATE 30

Wild variety of *N. acuminata* collected at Niles, California.
All drawings made from fresh material.

Fig. 1. An entire young plant. ×½.

Fig. 2. A typical flower, entire. ×2.

Fig. 3. The same split longitudinally, the ovary intact. ×2.

Fig. 4. The flattened face of the corolla limb. ×2.

Figs. 5 and 6. A half mature seed capsule. Fig. 5 natural size, fig. 6. ×2.

Fig. 7. A typical leaf occurring one-fourth the distance up the main axis. Natural size.

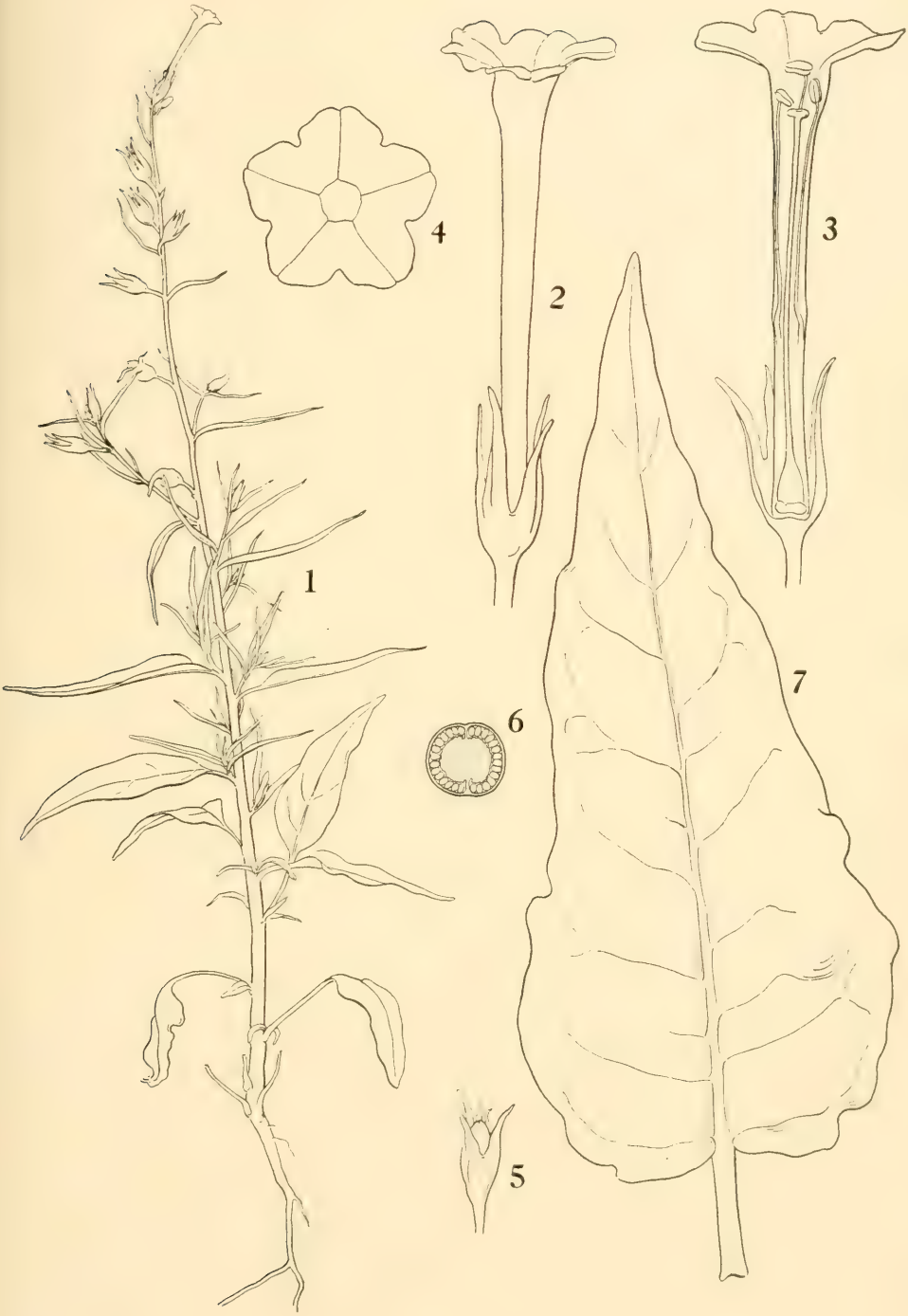
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PLATE 31

The number of flowers measured between October 2 and November 8 on single plants of varieties I, II and III of *N. acuminata* and of cross I ♀× III ♂, III ♀× I ♂, I ♀× II ♂, II ♀× III ♂, and III ♀× II ♂. In the case of the figure in the upper right-hand corner of the plate the number of flowers measured on all the plants representing the cross I ♀× II ♂ is shown.

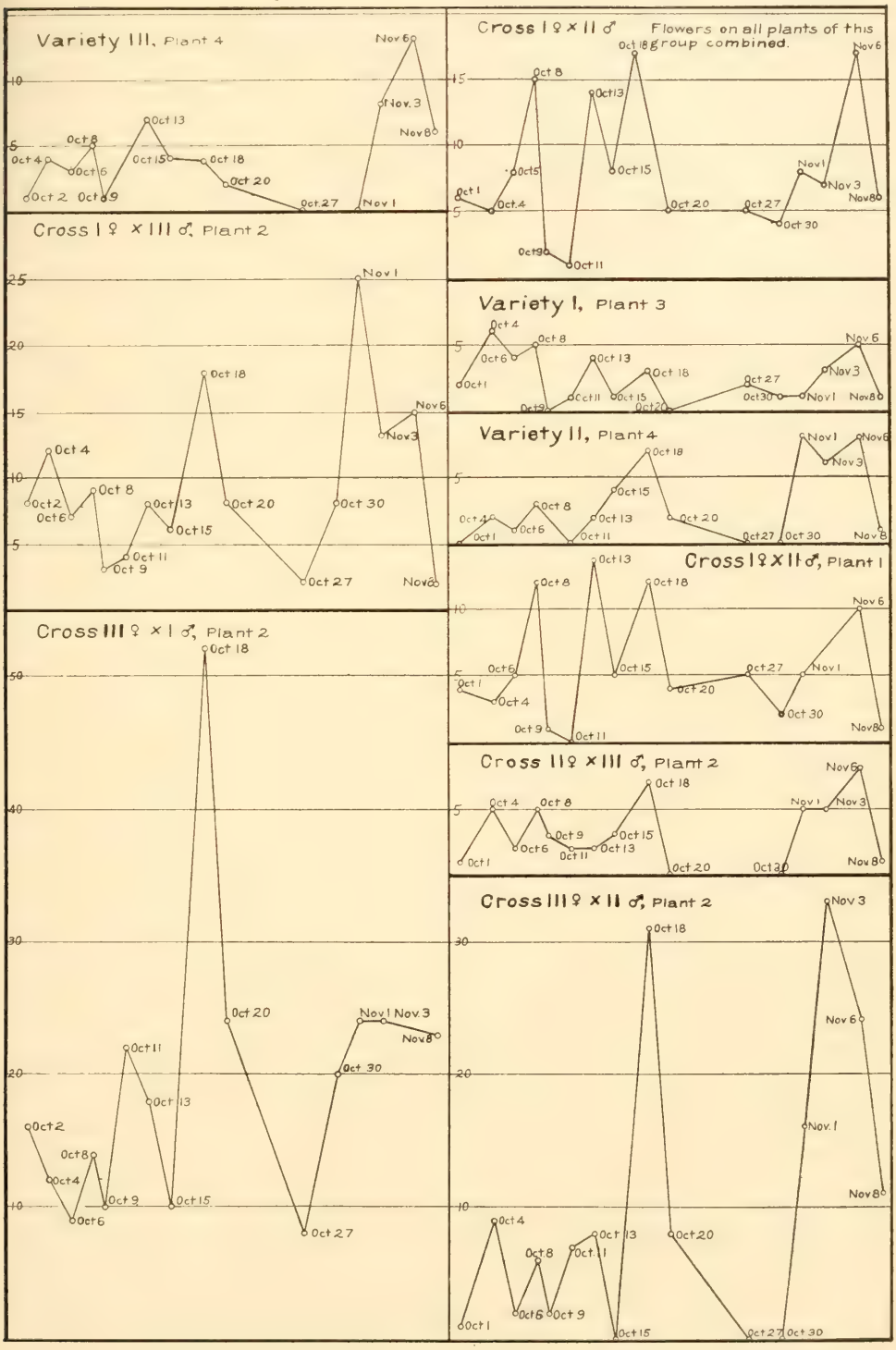
Variety III, Plant 4
Cross I ♀ × II ♂ Flowers on all plants of this group combined.
Cross I ♀ × III ♂, Plant 2
Variety I, Plant 3
Variety II, Plant 4
Cross I ♀ × II ♂, Plant 1
Cross III ♀ × I ♂, Plant 2
Cross II ♀ × III ♂, Plant 2
Cross III ♀ × II ♂, Plant 2

PLATE 32

The limits of fluctuation in corolla diameter of flowers on plants of varieties I, II and III of *N. acuminata* and of flowers on plants of the various crosses between these three varieties.

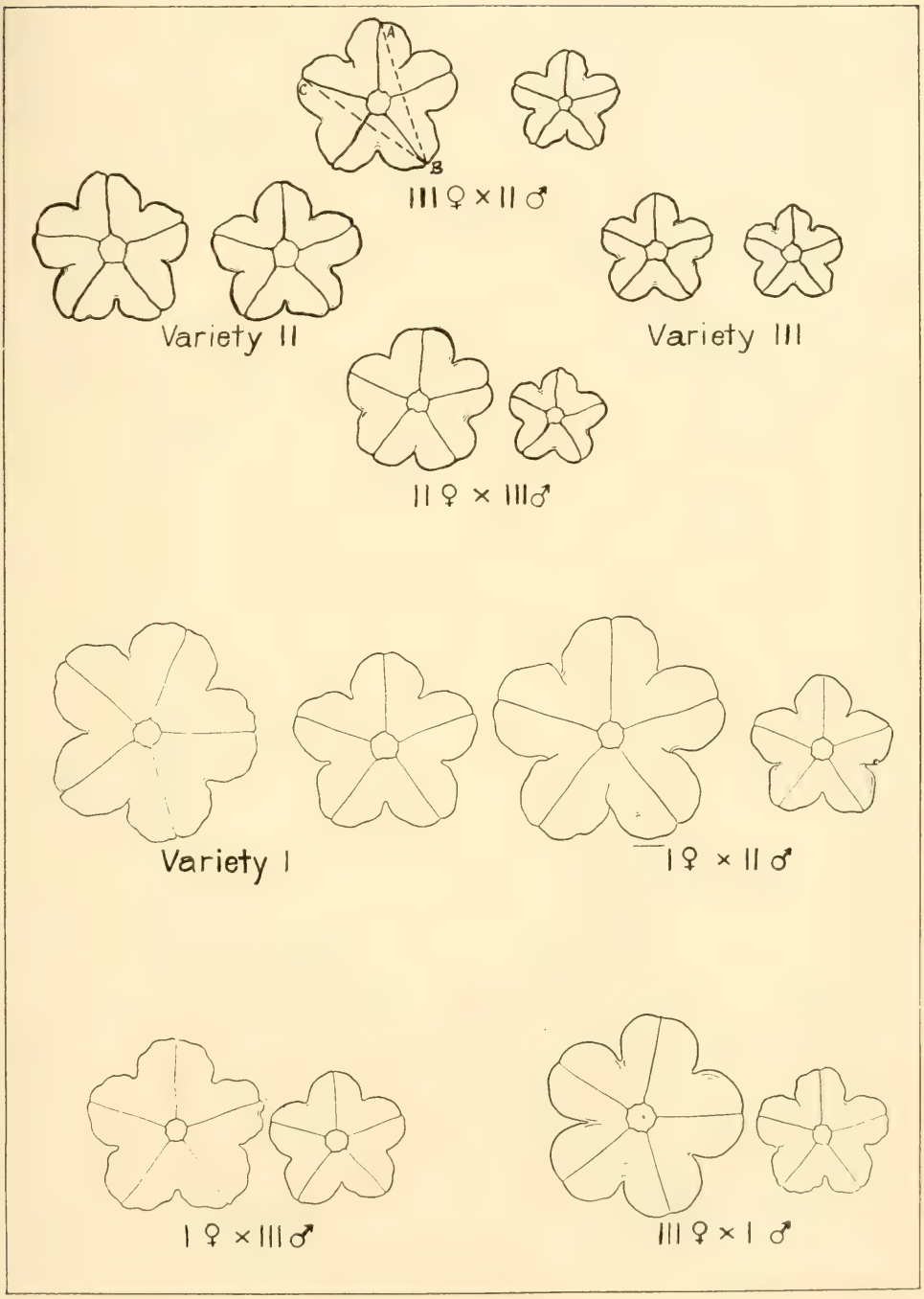
A
C
B
III ♀ × II ♂
Variety II
Variety III
II ♀ × III ♂
Variety I
I ♀ × II ♂
I ♀ × III ♂
III ♀ × I ♂

PLATE 33

The degree of fluctuation in corolla diameter of flowers on plants of variety I, variety III and of flowers on plants of the cross III♀× I♂, on each of sixteen different days between September 15 and November 6. The heights of the columns represent the relative number of flowers measured, of the various diameters noted as occurring in the flowers of the two varieties and the cross between them.

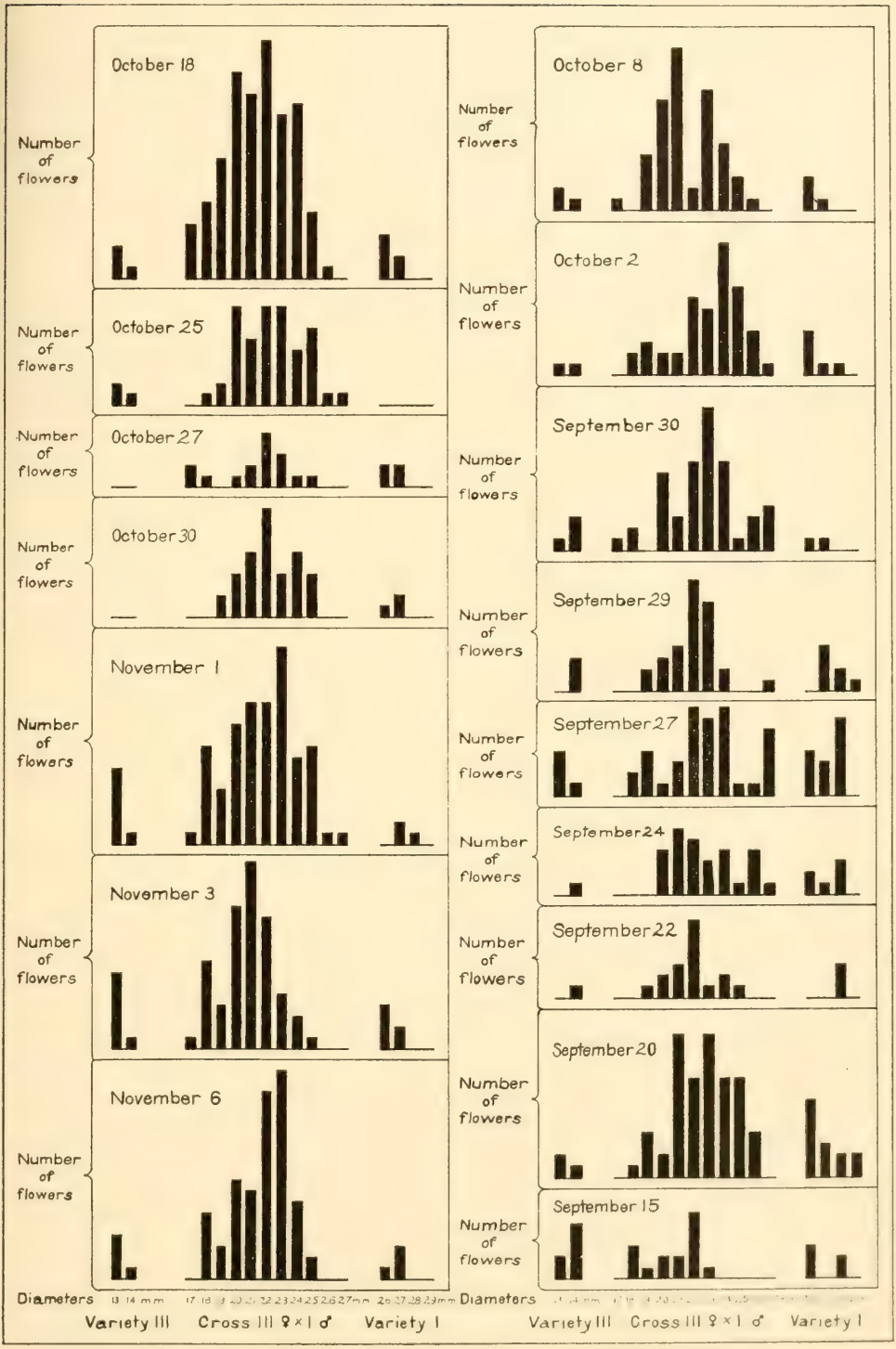
October 18
October 25
October 27
October 30
November 1
November 3
November 6
October 8
October 2
September 30
September 29
September 27
September 24
September 22
September 20
September 15
Number of flowers
Diameters
Variety III
Cross III ♀ × I ♂
Variety I

PLATE 34

Fig. 1. F_2 tobacco seedlings of hybrids between *Nicotiana Tabacum* variety *macrophylla* and *Nicotiana Tabacum* variety *virginica*. On the left seedlings grown from light seed, on the right seedlings grown from heavy seed. Many of the seedlings grown from light seed are one month older than the seedlings grown from heavy seed.

Fig. 2. Typical flowers of three varieties of *Nicotiana acuminata* showing the difference in corolla diameter. 150/07 is variety I, 192/08 is variety II and 53/03 is variety III. All approximately $\times \frac{1}{2}$.

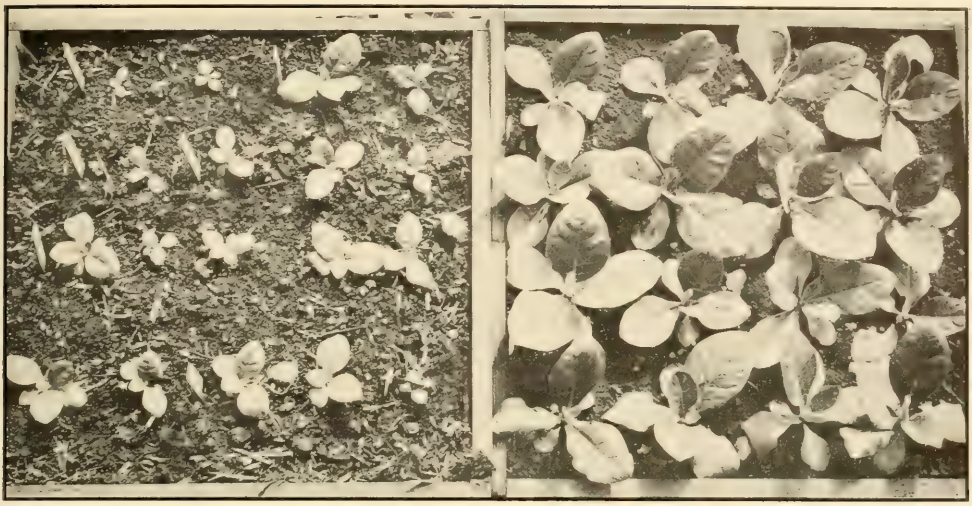

Fig. 1

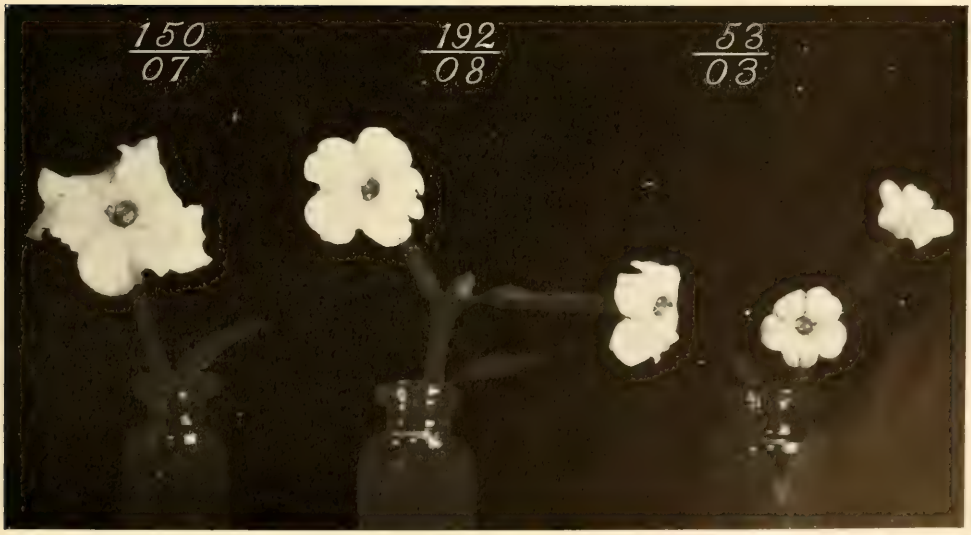

Fig. 2

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QUANTITATIVE STUDIES OF INHERITANCE IN NICOTIANA HYBRIDS. II.

BY

THOMAS HARPER GOODSPEED

A preliminary report on the inheritance in F_2 of flower size in hybrids produced from three varieties of *Nicotiana acuminata,* and notes on certain sterile hybrids of *N. Tabacum* varieties and *N. sylvestris* together with remarks on the present status of the "unit character" conception in studies of heredity.

In a previous report (Goodspeed, 1912) on the inheritance of flower size in F_1 hybrids of *Nicotiana acuminata* varieties the following points were noted: (1) the parental varieties, (varieties I, II and III) bore flowers during two seasons which showed small fluctuation in corolla diameter and were definitely set apart from one another in respect to this character, i.e., there was no intergrading of one flower size variety into another; (2) the parental varieties were distinguished from one another almost solely in respect to this flower-size character; (3) the results of the measurements of flower size in F_1 showed that the fluctuation in corolla diameter was practically twice as great and often nearly three times as great in this generation as in the pure bred parents grown in either of the two years during which their flowers were measured, and (4) the measurements of the corolla diameters of the flowers on the plants of each group of hybrids gave an average diameter for each group which was practically the same as the calculated average between the corolla diameters of the corresponding two parents—i.e., each of the five average

hybrid corolla diameters formed intermediates in size between the corolla diameters of the parents of the corresponding cross. A suggestion which was advanced with reference to this situation in F_1 was that "the occurrence of large flowers, small flowers, and flowers the corolla diameter of which show every degree of gradation between large and small in F_1, of a cross between a large flowered and small flowered variety of *N. acuminata*, represents the maximum degree of segregation which occurs in such a hybridization." A tentative suggestion as to F_2, also made there, was as follows: "The F_2 plants grown from the seed produced by self-fertilizing the flowers of this hybrid may give us a few plants bearing flowers the corolla diameters of which show small variation in size. Succeeding generations may reduce the variation still further until we have regained either one or both of the parental varieties which entered into the production of the F_1 generation or have established a new flower size variety which will thereafter come true to a certain diameter of flower which varies only slightly in size."

During August, September and October of this year (1912) over five thousand measurements of corolla diameter of flowers on the parental varieties and F_2 hybrids have been made. The results of these measurements make plain the necessity of observing the behavior of F_3 individuals before these F_2 results can be accurately commented upon. In addition it seems desirable to obtain measurements upon the flowers of the following types of *N. acuminata* plants in 1913 (1) the F_1 hybrids of 1910 grown in the field again and also under various controlled experimental conditions; (2) the F_1 hybrid remade in 1912 and grown under the conditions stated in (1); (3) the parental varieties to be produced from 1909, 1910, and 1911 seed and grown under these same conditions; (4) F_2 generation plants brought to maturity in the greenhouse; and (5) the obtaining of hybrid seed in the greenhouse from the parental varieties therein grown and the production from such seed of F_1 plants some of which will be grown in the field and some in the greenhouse. The value of certain of these desiderata was not appreciated at the start of the growing season just past and in the other cases lack of room and other facilities made their satisfaction impossible. The data

that may be accumulated from the above outlined material together with that which is at present available should go as far as it is possible to go toward settling the significance of fluctuating variability in the experimental results thus far obtained and in those that it is hoped may be obtained in succeeding hybrid generations (cf. also, Shull, 1902 and 1904, Tower, 1902 and 1906 and Love, 1910 and 1911). The importance of this factor has been better appreciated this year (1912) during which the parental varieties have been grown in a different situation and have exhibited considerable increase in individual flower size and a slightly increased fluctuating variability. The present paper is thus presented merely as a preliminary report which it is hoped will give a hint as to the various lines along which the problem involved is to be approached and also serve as the vehicle for the presenting of certain views concerning a problem of somewhat larger significance.

As will be seen by reference to an earlier paper (*loc. cit.*), during the seasons 1910, 1911 the measurement of flowers on varieties I, II and III showed that their mean corolla diameters were respectively 27, 20 and 13 mm. with fluctuations never exceeding two millimeters either greater or smaller than the mean diameter in each case. Thus variety I bore flowers having corollas with diameters from 25 to 27 mm., variety II bore flowers having corollas with diameters from 19 to 22 mm. and variety III flowers having corollas with diameters from 13 to 15 mm. The measurements in 1912 give the following results: Variety III varied in corolla diameter from 15 to 18 mm., with the mean diameter approximately 16 mm., Variety II showed fluctuations between 22 and 26 mm., with the mean diameter approximately 24 mm., and Variety I bore flowers the corolla diameters of which showed extremes of fluctuation at 30 mm. and 35 mm. with the mean diameter approximately 32 mm. The size of the flower has thus been increased throughout and the fluctuation in corolla diameter also increased as compared with the results of the previous two years measurements. The plants, parent and hybrid, of *N. acuminata* were grown on higher, better drained, less devitalized land and obtained a maximum of sunlight and heat (cf. Goodspeed, *loc. cit.* p. 123). The plants were spaced in the

rows and cultivated just as in the cultures of the previous two years. The figures given are most important, however, in that they show that with an increase in size and fluctuation due to more favorable cultural conditions no intergrading of one variety into another takes place and that on the contrary the three varieties are even better distinguished from one another in corolla diameter than before.

The following F_2 hybrid generations have been raised during 1912—F_2 of the crosses III♀×II♂, I♀×II♂, II♀×III♂ and III♀×I♂. Over five thousand measurements of corolla diameter have been made upon three of these F_2 generations while the fourth is at present just coming into flower. From 15 to 18 plants in each group were brought to maturity and their flowers measured.

The actual results (calculation of the means and coefficients of variation) of these measurements have not as yet been completely determined but the general trend of the results is sufficiently clear to make this preliminary account of them possible. Contrary to the expectation expressed the variation in corolla diameter in F_2 appears to be more or less greatly increased as compared with that noted in F_1. Thus, whereas in the F_1 plants the range of variation was two or three times as great as that of the parents, in F_2 the minimum range of variation is always over twice and the maximum range five or six times as great as that of the parents in 1910 and 1911. In the case of F_2 of the cross I♀×II♂ certain plants show a range of variation in corolla diameter from a flower 2 mm. smaller than the smallest flower ever measured on Variety II to a flower 3 mm. larger than the largest flower borne by Variety I in 1912 and 7 mm. larger than the largest flower produced by Variety I in 1910 and 1911. An increase in vigor where size and form characters are being dealt with, is often to be expected in F_1 yet this common result of hybridization was not observed in the F_1 previously reported upon (cf. also, East and Hayes, 1912). Certain plants in the F_2 generation of this same cross, however, exhibit as small ranges of variation as do the parental varieties this year (1912) and they are always the plants which bear the largest flowers. F_2 individuals of the cross III♀×II♂ similarly exhibit an extended

range of variation in corolla diameters but in this hybrid the upper limit of variation of the 1912 variety II is never exceeded in the flower of any individual though the largest sized flower is 4 mm. larger than the largest flower on variety II in 1910 or 1911. It is also to be noted that in the F_2 of this cross there are no individuals which exhibit a small range of variation such as was found in certain plants in F_2 of the cross I♀×II♂. Every plant bears flowers smaller than the smallest flowers ever measured on the small flowered parental variety III and every plant also bears flowers larger, with possibly one exception, than the smallest flower measured on Variety II in 1910 or 1911. In other words the F_2 plants from this cross exhibit identical ranges of variation with respect to the corolla diameters of the flowers which they bear.

Averages of corolla diameter have, in none of the cases, been calculated. The results at present available and as outlined above seem, however, to preclude the possibility of a simple Mendelian interpretation of the inheritance of the flower-size characters peculiar to varieties of *N. acuminata.* Some difference of opinion is at present apparent with reference to the interpretation of the mode of inheritance of morphological characters—size and form characters. Certain cases of blending inheritance in F_1 are seemingly not followed by the occurrence of segregation in F_2. On the other hand the method of interpretation of certain otherwise obscure experimental results as given by Nillson-Ehle (1909), East (1910), Hayes (1912) and others seems to some to open the path for the explanation of an almost limitless range of experimental results, derived from a study of the inheritance of morphological characters, on what seems to be a modified Mendelian basis (cf. Hayes, 1912, p. 34). Thus the occurrence, as outlined above, of a seemingly greater degree of variation in F_2 than in F_1—and the truth of this situation is open to question until the true meaning of fluctuating variability in our experimental material is more thoroughly determined—appears to make it plain that segregation does occur (Castle, 1911, p. 137) or, as one writer has so aptly put it "the segregation of *potential* (italics mine) characters in the germ cells of hybrids and their chance recombination in later generations" (Hayes, 1912) has

actually taken place. The point brought up in this connection is, to the writer's mind at least, of somewhat broader significance than the mere fact that certain experimental results in F_2 may or may not be susceptible of a particular interpretation. We are involving, in Mendelian interpretations of variation which are apparently continuous, the old question of what is a "unit-character" (cf. Darbishire, 1911, p. 131).

In 1909 when this breeding experiment with flower size varieties of *N. acuminata* was suggested to me by Professor Setchell, the facts of size and form inheritance had not received the amount of attention which has more recently been accorded them. For this reason, perhaps, the crossing of a large flowered form with a small flowered form was taken in good faith as likely to involve a single Mendelian pair of characters and a simple mono-hybrid ratio was anticipated for the results of measurements of corolla diameter of flowers on F_2 individuals. When one bears in mind that the parental varieties are in general indistinguishable one from the other except on the basis of this corolla diameter character and that the greatest variation in corolla diameter of their flowers has not exceeded 6 mm. during three consecutive years (on the basis of over 3000 measurements) it is not strange that orthodox "unit-character" behavior was looked upon as the basis for the Mendelian ratios which were vaguely anticipated. Nothing, to the writer's mind has been—and still is in some quarters—so firmly fixed in the "genetical" mind as the "Law of Dominance," the "Law of Segregation" and the "unit character" conception. The "Law of Dominance" never was a law and is "no inseparable attribute of Mendelian inheritance" (cf. Bateson, 1909, p. 13 and p. 53). This seems to be generally recognized at present, so thoroughly recognized indeed that the possible deeper and broader significance of "dominance" in F_1 in general is in danger of being almost entirely overlooked. With reference to the "law of segregation" a leading student of the problems of heredity has recently said "there can be no reasonable doubt that Mendel's law is of fundamental importance in genetics" (Castle, 1912, p. 352). And the most important contribution of Mendel's discovery to the study of genetics is the assumption that "segregation of potential characters in the germ

cells of hybrids and their chance recombination in later generations'' (Hayes, 1912, p. 28) does take place. In 1909 I think it may be safely stated that, broadly speaking, the ''unit-character'' conception had become ''an inseparable attribute of Mendelian inheritance.'' The literature dealing with the problems of heredity had become so permeated with the unit-character criterion that it was difficult to discuss a hybridization experiment without reference to the more distinct morphological or physiological attributes of an organism by the use of this term. At the present time we are led to believe that there are no ''unit-characters'' in the sense of ''units distinct and indestructible which may meet in fertilization but separate again at the formation of gametes'' (Castle, 1911, p. 38). Such an attitude may be one which is entirely in harmony with the progressive spirit of the times—i.e., the rapidity with which speculation along Mendelian lines has swept us forward.

The question concerning the ''relative constancy of unit-characters,'' perhaps because of ''its illusiveness'' and certainly because the experiment was undertaken in the belief that unit-characters were ''transmitted as independent units in inheritance'' (Darbishire, 1911, p. 216), has certainly taken on ''a perennial habit'' (East, 1912, p. 644) with reference to the behavior of flower size characters in *N. acuminata* hybrids. The occurrence of interesting and seemingly important evidence on the divisibility of what we may call a strictly ''physiological unit-character'' together with the complexity of the ''flower size unit-character'' problem was the occasion for the preparation of a note on the present status of the unit-character conception which was going to press when Professor East's recent paper (Amer. Nat. 46, 551, p. 663) came to hand. While it is recognized that the greater part of the whole situation has been reviewed and commented upon by East (*loc. cit.*) and Castle (Amer. Nat., 46, 546, p. 352) it seems nevertheless advisable to present a brief outline of the above mentioned note together with such experimental evidence, bearing immediately on the subject in hand, as was therein contained.

It was pointed out that Mendel chose the experimental material used in the most famous experiment with a view doubt-

less to meeting some such list of technical requirements as is given by East and Hayes in a recent paper (1911, p. 6). Perhaps all of the first six of the desiderata there stated may have influenced Mendel's choice but undoubtedly the first was most prominently in his mind—i.e., "the genus or species under investigation should be variable. There should be a goodly list of types which are differentiated by definite characters easy of determination" (*loc. cit.*, p. 6). The genus Pisum was variable and supplied a goodly list of types which were differentiated to Mendel's mind by very definite characters. These definite characters later investigators have chosen to speak of, often rather loosely, as "unit-characters" (cf. Darbishire, 1911, p. 131).

Such definite characters which seem to be "unit-characters" and to be transmitted as "independent units in inheritance" have many times been dealt with in the past and will continue to be dealt with in the future. For when "definite characters are sufficiently constant to be expressed by a fixed standard . . . the whole heredity short-hand is . . . simple" (East, 1912, p. 648). On the other hand there are quantitative characters which are so inherited that their mode of inheritance can only be explained in accordance with the "Mendelian notation" by the assumption that within the character a "multiplicity of factors exist, each independently inherited and capable of adding to the character in question." The "philosophical query as to whether the characters of the organic complex of which living organisms are composed can in any sense be dissected and analyzed into the units of heredity which are the basis of Mendelian inheritance" seems out of order at this point.

The fact remains that some real and definite distinction has been made in the past and will be made in the future, so far as an actual hybridization experiment is concerned, between characters which are sufficiently definite to be represented in the germ cells by hypothetically fixed determiners or genes and characters, mostly quantitative, which are heritable potentialities only and are represented in the germ cells by independent and interchangeable units functioning to produce addition, and thus seemingly subtraction, phases of the original character. To the writer's mind this distinction has been brought to light, and will

be maintained for a time only, simply by reason of the distinction in the method of accumulating the experimental results, between the experiments where quantitative and in those where qualitative characters are being dealt with. Experimental results seem to have established a distinction between characters which appear to be tangible realities in pure bred parental strains, "atomic," and which can be spoken of as "unit-characters"—i.e., "units distinct and indestructible which may meet in fertilization but separate again at the formation of gametes" (Castle, 1911, p. 38), and characters which appear to be just as tangible realities in the parents but are really "molecular" in structure, capable of infinite divisibility and, as characters, really exist in the parents themselves in possibility but not in reality—i.e., as potential characters.

With reference to the divisibility of what might be called a "physiological unit-character" an interesting bit of experimental evidence has recently appeared among the *Nicotiana* cultures in the Botanical Garden of the University of California. In 1910 and 1911 a number of cross pollinations, back and forth, between *N. sylvestris* and a number of *N. Tabacum*-varieties were made by Professor Setchell. Ten groups of hybrids, the results of these crosses, are growing this year (1912). Among them are those which involve *N. Tabacum* var. *macrophylla* (U.C.B.G. 22/07), (Setchell, 1912, p. 8), *N. Tabacum* var. *calycina* (U.C. B.G. 110/05) (*loc. cit.*, p. 6), *N. Tabacum* "Maryland" (U.C. B.G. 78/05) (*loc. cit.*, p. 5), etc., with *N. sylvestris* (U.C.B.G. 107/01) (*loc. cit.*, p. 29), as either the male or female parent. These hybrids, in each case, are practically sterile, at least as compared with the heavy seeding character of the parents. The flowers, in most cases, fall a short time after anthesis, the pollen is more or less scanty and in general they are of the type of hybrids of various species of *Nicotiana* with *N. sylvestris* which have previously been reported to be completely sterile (cf. Baur, 1911, p. 224, East and Hayes, 1912, and the literature there cited).

It has been noticed in all the *N. Tabacum*-varieties and, to a lesser extent, in *N. sylvestris* that as an inflorescence, consisting often of from 40 to 50 flowers, passes from the flowering to the seeding stage a few flowers will often appear from flower buds

that have developed late in the season at the base of maturing seed capsules. The majority of these buds, and numbers of them may be formed, die and fall but in certain cases 4 to 10 may develop into weak, stunted, at times abnormal, flowers. The appearance of these flowers is rather striking, rising as they do hardly above the tips of the brown and almost mature seed capsules about them, with the corolla tube so shortened that the stigma stands 3 to 4 mm. above the flattened limb which limb is much reduced, flaccid and early withering. This situation very probably depends upon the physiological balance between the metabolic activities which look to the production of flowers and those which have to do with the maturing of the seed formed from these flowers. Some data are at hand which seem to show that on a given flowering shoot of the indeterminate type the size of the flowers which it bears is, roughly speaking, inversely proportional to the number of seed capsules which are allowed to mature. The agricultural practice of "topping the bald sucker" and "suckering" tobacco plants is merely an effort, and apparently an effective one to restrict the growth and especially the metabolic activities of the plant body to the vegetative structures as opposed to those strictly reproductive. The reactions normally proceeding in one direction are forcibly reversed or perhaps reaction products are not allowed to accumulate in the usual regions and the metabolic reactions reach a point of equilibrium at a later period and in another part of the plant. Similarly what is recognized as a periodicity in the production of flowers means the concentration of the products of metabolic activity in different regions at different times to accomplish special and individual results. The conditions, in the case of a large old inflorescence maturing seed, are not such as to allow of the production of normal, vigorous flowers—they are out of place and the constructive materials for their proper development are not present in sufficient quantity. Phenomena, as closely correlated as these, so dependent upon broad, general physiological states common to all plants are not ordinarily thought of as "unit-characters," and, yet, on the other hand, they might seem to be the fundamental, truly indivisible "unit-characters" on the basis of which our experimental mingling of less fundamental, heretible

peculiarities is possible. A plant that is making seed cannot produce better than stunted, functionless flowers among the maturing seed capsules yet if the young seed capsules are removed as they form, the secondary flower buds will produce normal flowers almost indefinitely. It is a repetition of the "topping" and "suckering" under different conditions to accomplish the same type of ends and a rather perfect set of cause and effect phenomena.

With these facts in mind it was a surprise to find, in the case of the hybrids between *N. sylvestris* and *N. Tabacum*-varieties, that on an inflorescence, and usually with all old flowers fallen and the stems bare, these same late flowers were as thoroughly stunted as in the case of the parents. Five to eight flowers on these older hybrid inflorescences, on which no seed has been formed, often stand on the naked branches and are as abnormal, stunted and poorly formed as in the parent plants. In other words though no subtraction takes place from later flowers in favor of maturing seed capsules in these hybrids, still the phenomenon of reduced reproductive parts is apparent in these later flowers. Here we have a "physiological unit-character" dependent upon metabolic activities and one which is seemingly an indivisible unit connected with cause and effect relations common to all plants which, nevertheless, is so split up in crossing that the effect is inherited when the cause is absent. Undoubtedly the real situation here is too obscure to offer more than an interesting field for speculation but the fact seems of sufficient interest to be noted. In the parent plants it is possible to eliminate the effect (reduction in size and vigor of older flowers) by eliminating the cause (removing young seed capsules as they form)—in the hybrid individuals the cause is self, automatically, naturally removed but the effect is present unchanged.

An explanation of this phenomenon, which has hardly more than the minimum of experimental evidence as its basis, should, however, be mentioned here. It is just conceivable that the factor for the production of abnormally reproductive organs represents a dominant character in these hybrids commonly considered to be completely sterile and which normally are

undoubtedly partially sterile. This supposition is supported by the fact that generally only a few axillary buds are found to be produced at the bases of maturing seed capsules in the *N. sylvestris* parent and that where a flower does develop from such a bud reduction in its size, noted under similar conditions in the *N. Tabacum* parental varieties, is not so striking. The factor introduced by the *N. sylvestris* parent thus may stand for the recessive condition of this abnormal-flower character. The normal production of seed may, also, be looked upon as a separately inherited tendency which in these sterile hybrids appears to be in a latent condition or possibly represents the recessive member of another Mendelian pair with reference to these hybrids. Though it may not be profitable to push the analogy too far it is to be noted in this connection, that *N. sylvestris* has the very interesting tendency, strongly emphasized in our cultures, of reproducing "vegetatively" from the roots year after year and that the hybrids made with *N. sylvestris* as one or other parent also possess quite markedly this peculiarity which is rather unusual in the genus *Nicotiana* but is common in other genera of the Solanaceae. Thus, following out the analogy, it may be possible that sexual reproduction vs. its absence (vegetative reproduction) constitute the members of a Mendelian pair and that the absence of vigorous seed production in these hybrids represents a recessive condition. While the experimental basis for these suppositions is rather vague the facts brought out in connection with them may prove to be of some importance with reference to the well recognized sterility of hybrids in which *N. sylvestris* is involved as a parent. In this connection it may be said that a considerable variety of experiments are at present being carried on in the effort to bring about normal seed production in the case of these "sterile" hybrids and also that a quantity of cytological material has been collected from them and from the parental varieties in the hope that a considerable mass of data on the nature of sterility in such hybrids may be at hand before the end of the coming year. The above, in general, is interesting principally in that it adds weight by analogy to the point of view which looks upon the "unit-character" not as an "atom" but as a "molecule" or indeed as a heritable potentiality

capable of infinite divisibility and innumerable states of semi-union with other correlated potentialities.

All experimental studies in the physiology of heredity have shown the necessity of consciously allowing a hypothetical term to take the place of any visible outward expression of a certain tendency—physiological in practically every instance—peculiar to or inherent in an organism. This hypothetical term is demanded primarily for the formulation of a mathematical expression which can then express a multitude of situations involving the tendency in question. This hypothetical term is secondarily of real value in that it serves as a short-hand expression for this same tendency which may be utilized in referring thereto in a considerable number of connections where mathematical expression is not called for. It is, however, perfectly evident that this hypothetical term has by reason of its association with the living tendency to serve the above two ends, gained absolutely nothing in the extent to which it actually represents a tendency, peculiar to or inherent in a living organism. This has many times been pointed out by East and others. The fact, on the other hand, remains that the accumulation of experimental results in the past 12 years that have been more or less readily susceptible of interpretation according to the Mendelian notation has resulted in a situation wherein hypothetical terms have absolutely overshadowed the tendencies dealt with. The distinctions between qualitative characters and quantitative characters rest upon as hypothetical a basis and involve the use of hypothetical terms as surely as does the use of such clearly hypothetical expressions as unit-character, factor, or gene. This is evident in that we now know that certain qualitative characters and the explanation of their mode of inheritance may rest upon the same assumption or series of assumptions which originally was suggested primarily to explain the facts concerned in the inheritance of quantitative characters. The more carefully and "quantitatively" the inheritance of qualitative characters is investigated, the greater will be the field for the application of the assumptions involved in Mendelian interpretations of variations which are apparently continuous, if the experimental results can hope to be explained according to the Mendelian notation. In other

words, a great assumption was made in the early years of Mendelian interpretations in that the hypothetical term unit-character was assumed to represent a simple, indivisible, more or less independent and dependable entity and it was this *unit-character* that was expected to reappear unaffected in F_2. Does not the perennial character of the question relating to the stability of the unit-character depend simply upon the unwillingness or inability to appreciate the fact that blackness is as hypothetical a something as the term "unit-character" which is applied to it? The fact that blackness behaves as an orthodox Mendelian dominant makes the heredity short-hand simple and also seems thoroughly to obscure the fact that three factors may fundamentally be responsible for the outward expression of the black tendency just as truly as that three factors are necessary for the production of the purple color in the aleurone cells in certain varieties of maze. Equally truly it is conceivable that 30 factors may be concerned in this purple maize color tendency if 30 factors, units or genes are found to be responsible for the outward expression of flower size in a large flowered form of *N. acuminata.* Black, purple and large flower size are equally involved in this situation. In other words whereas blackness has apparently been shown to be an entity, sufficiently a unity to remain unchanged in hybridization, the true significance of more fundamental units underlying purple aleurone color, height of plant, number of rows, length of ear and size of seed in maize, and responsible for various fruit sizes, number of leaves in tobacco, etc., has been made apparent by the same means. In other experimental material, however, each of these characters may in time be shown to behave as a simple Mendelian dominant also. It seems probable that in time the majority of characters, both "qualitative" and "quantitative," may be found to be modifiable under selection. Each individual addition or subtraction stage will "Mendelize"—using this term to signify experimental results in accord with the Mendelian notation in its present expanded condition—with the same pure line individual in each instance. It has been shown that each may behave as a simple Mendelian "recessive unit" (cf. Castle, 1912, p. 356) or each may so behave that a multiplicity of more fundamental interacting units

or genes must be assumed as the basis of the character appearing in modified condition in each stage. Considerations of this sort simply serve to show that certain characters are sufficiently stable within themselves so that the fundamental interacting units upon which they depend for their outward, somatic expression, do not appear while other characters, not so stable, under the stress of hybridization do manifest the fundamental units through the interaction of which they are able to appear in substantial form. The fact that they are manifested in one case implies that fundamentally they, though not apparent, are present in all other cases also. So soon as it can be demonstrated that for any one character the influences upon which it depends, the factors beneath the surface, are actually apparent, in that hypothetical terms to stand for them in mathematical expressions can actually be proposed, at once one postulates for all characters the existence of similar influences whether they are similarly apparent or not. In other words, all "unit-characters" are "molecular" in structure. Aggregates of similar "units or genes" evidently are responsible for the outward evidence of "unit-characters" and the unity of this "unit-character molecule" may depend upon the degree of affinity of the units or genes—a true chemical affinity perhaps (see East and Hayes, 1912, p. 35). The more quantitative our investigation becomes, the more clearly is brought out the looseness, rarely the firmness, of the bond which holds the innumerable units or genes to the "unit-character" conception. From the Mendelian standpoint it seems clear that we are getting below the surface with reference to the real significance of the "unit-character" conception. The presence of more fundamental, basic heritable influences within the seemingly indestructible "unit-character" is assumed in order to make the mode of inheritance of both "qualitative" and "quantitative" characters susceptible of interpretation according to the Mendelian notation. All "unit-characters" must then be assumed to be alike in the possession of these fundamental, "subepidermal" influences whether their presence needs to be assumed or not. Otherwise we have to recognize two categories (1) "unit-characters"—distinct, atomic, indestructible and (2) potential characters—molecular in structure and dependent upon the interaction

of independently heritable units for their somatic expression. These interacting units may be looked upon as the substances entering into a reaction which is represented at a point of equilibrium by an outward, visible, seemingly or truly indestructible "unit-character" peculiar to an organism. The individual reacting substances are separately inherited but may be so linked (a chemical affinity) in the case of certain characters that the same reaction will reach the same point of equilibrium time after time and we then appear to be dealing with a unit distinct and indestructible. Such analogies (cf. in this connection Moore, 1910 and 1911) supply a limitless field for speculation yet it may be interesting to note that increase of size and precocity of somatic characters due to heterozygosis might be concerned with an increase in the rapidity of growth reactions caused by an increase in amount of reacting sustances. An attitude in general analogous to the above is taken by East (1912, p. 648) with reference to the changes in the expression of a character under the stress of what he refers to as "modifying conditions both external and internal"—"when external we recognize their usual effect in what we call non-inherited fluctuations, when internal we recognize their cause in other gametic factors inherited independently of the primary factors but modifying its reaction during development." In so far as this is an assumption which seems to postulate a limitless extent of organic complexity as the fundamental basis for those tangible characters we seem to see in an organism and draws an analogy between chemical reactions and the interrelations of these "factors," the above is "a physiological conception of heredity." On the other hand if it is to become a question as to how many separately inherited tendencies contribute to the entity which we loosely speak of as a "unit-character" the logical end of the inquiry will be found to lie in a biochemistry of the individual cell (cf. Czapek, 1911, ch. 10).

It is well to bear in mind in connection with such elusive considerations as these that as we "bid farewell to the broad daylight of observation" we at once proceed to "enter the dark and treacherous alleys of inference." Observation, however, has resulted in the accumulation of certain experimental results the

interpretation of which according to the Mendelian notation is only possible after a certain number of assumptions have been indulged in. My point is simply that if in one case it is necessary to assume an interaction of units within a certain "unit-character" then within all "unit-characters" we must recognize similar fundamental units whether the mode of inheritance of the character in question calls for such an assumption or not. On this assumption that all characters are molecular in structure, representing aggregates of units each of which is capable of modifying the character being dealt with, we have a basis for the explanation of all such considerations as those presented by Castle (1912). On this basis there is nothing more inconstant than the individual characters peculiar to an organism except the organism itself which represents the sum of these individual characters. On this basis again the range of results capable of interpretation according to the Mendelian notation is limitless and there can be no question of stretching the Mendelian notation to cover any particular case for, by reason of its assumptions, it has become immeasureably extensible.

The question then arises to what extent are these primary assumptions valid or in other words does the end justify the means. The end is nothing more important—with reference to the limits of our present knowledge of the phenomena involved—than the natural desire and valuable effort to interpret all experimental results in accordance with a notation which has been found to apply to a relatively small group of characters—probably less than three hundred characters peculiar to domesticated animals and plants. The means employed to accomplish this end are beginning to involve the complication of the mathematical expressions used to such an extent that nothing can actually be gained for the practical breeder and the necessity of raising and examining the extremely large F_2 and F_3 generations demanded will restrict independent investigation immensely. Indeed it seems hardly in accord with the "progressive spirit of the times" and the growing recognition of the immensity of the problem involved, to demand a strict conformity of effort and interest. The value of the Mendelian notation as a *generalization* certainly fails if it is true that not more than a few hundred

characters, the great majority of which exist in a state of domestication, are inherited in Mendelian fashion (cf. Darbishire, 1911, p. 239). On the other hand these same few hundred characters may represent almost the sum total of those which are of interest to the practical breeder. When the breeder finds that he is dealing with a Mendelian dominant *his* heredity "short-hand" is simpler than it ever could have been without the Mendelian notation. If the inheritance of the character he is interested in is more complex—i.e., if it is a "bulk character"—he will follow such obvious suggestions as those given by Castle (*loc. cit.*, p. 362). In either case the breeder will probably be primarily interested, just as he always has been, in only one thing—i.e., increasing variability by crossing. The value of the Mendelian interpretation for the breeder has from the start been made apparent in practice and by no means all the characters which he is interested in and which will "Mendelize" have been dealt with at this time. Likewise the theoretical value for the science of heredity in the Mendelian point of view "that the contents of the germ cells and not the outward characteristics of the animals and plants dealt with must be our guide in breeding" cannot be overestimated. No one, it seems to me, can wish "to give up" the Mendelian point of view and its "handy and helpful notation" especially in those cases in which it is so handy and so helpful. On the other hand with reference to those cases in which, both for the student of the problems of genetics and for the practical breeder, the notation is neither handy nor helpful, though theoretically capable of application, a question must be answered—i.e., which is the way for progress?

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ON THE PARTIAL STERILITY OF *NICOTIANA* HYBRIDS MADE WITH *N. SYLVESTRIS* AS A PARENT

BY

THOMAS HARPER GOODSPEED

In 1910 Professor W. A. Setchell, in connection with other similar experiments, all of which have been made possible by a grant under the Adams Fund, made a number of cross pollinations back and forth between *Nicotiana Tabacum* var. *macrophylla purpurea* (U. C. B. G. 25/06) (Setchell, p. 10) and *N. sylvestris* (U. C. B. G. 107/01) (Setchell, p. 29) which species form a part of a considerable *Nicotiana* collection in the University of California Botanical Garden. The F_1 hybrid plants produced from the seed obtained as a result of these crosses were grown in 1911 and exhibited a striking increase in size (habit) and size of flower as compared with the parent plants (cf. East and Hayes, 1912). There seemed to be a possibility, also, that these hybrids might be only partially sterile, though as noted below no "pure" seed was formed under bag. On account of the interesting appearance of the 1910 hybrids in general and in particular with this suspected partial sterility in mind, all the *N. Tabacum*-varieties available, i.e., grown from pure seed in the pure line for a number of years, and one F_1 hybrid made between two such *N. Tabacum*-varieties, were in 1911 crossed back and forth with *N. sylvestris*. In the following list are given the hybrids produced from the seed of the 1910 and 1911 crosses with the hybrid numbers prefixed in each case.

1910 crosses.

H33—N. sylvestris × N. Tabacum var. macrophylla purpurea.

H34—N. Tabacum var. macrophylla purpurea × N. sylvestris.

1911 crosses represented this year (1912) by F_1 plants.

H35—N. sylvestris × N. (Tabacum) angustifolia (U. C. B. G. 68/07) (Setchell, p. 9).

H36—N. (Tabacum) angustifolia × N. sylvestris.

H38—N. Tabacum var. macrophylla (U. C. B. G. 22/07) (*ibid.*, p. 8) × N. sylvestris.

H40—N. Tabacum var. calycina (U. C. B. G. 110/05) (*ibid.*, p. 6) × N. sylvestris.

H41—N. sylvestris × N. Tabacum "Maryland" (U. C. B. G. 78/05) (*ibid.*, p. 5).

H43—N. sylvestris × the F_1 hybrid—N. Tabacum "Maryland" × N. Tabacum "Cavala" (U. C. B. G. 72/05) (*ibid.*, p. 5).

H44—The F_1 hybrid—N. Tabacum "Maryland" × N. Tabacum "Cavala"—× N. sylvestris.

H45—N. sylvestris × the F_1 hybrid—N. Tabacum "Maryland" × N. Tabacum "Cavala."

A detailed report upon these 1910 and 1911 F_1 hybrids is in preparation and it will only be said here that the 1911 F_1 hybrids duplicate in general the increase in vegetative and floral development noted for the 1910 series.

As will be noted in the above the reciprocals of only two of the five 1911 crosses were represented in the field in 1912. In the other cases viable seed was not obtained. This fact and the germination of the seed resulting from the various crosses made in 1911 are of interest in connection with certain results noted in a recent paper by East and Hayes (*loc. cit.*, p. 28). As will be seen there the seed produced from 18 crosses between *Nicotiana* species and between *Nicotiana* varieties which list includes the cross "*N. sylvestris* Speg. and Comes × *N. Tabacum L.*" and its reciprocal—germinated to the extent of 100 per cent. Which one of the numerous *N. Tabacum*-varieties was employed in this cross with *N. sylvestris* is not stated, but of the four varieties used in our crosses made in 1911 it is doubtful whether under the conditions employed in our cultures (Goodspeed, 1912, p. 132)—and these are the conditions commonly available in similar work—the seed resulting from any of these crosses of ours germinated to the extent of 100 per cent. Results are at hand for

germination tests of parent and hybrid tobacco seed involving over 22,000 seeds and including over 10 species and varieties of *Nicotiana* (Goodspeed, 1913 (2)). These germination tests deal with the seed taken from approximately 60 different plants. It was possible to fairly well control the conditions under which germination took place and the arrangement of the seed to be germinated was such that a minimum error for the observations can be claimed. In only one case, however, employing duplicate tests for the seed of each plant, did 100 per cent germination take place. In the case of the seed of one other plant one of the two tests of 100 seeds each germinated 100 per cent, but in the other test of this same seed only 97 per cent germination took place. Thus there seems to be some reason to doubt whether 100 per cent germination is normal in the case of seed produced by *Nicotiana* species and varieties or by the hybrids made between them. Unfortunately it has not at this time been possible to test the germination of the seed produced by the crosses between *N. sylvestris* and *N. Tabacum*-varieties mentioned in the above tabulation. In general, however, it is well to understand what is meant when germination percentages are given.

There are a number of references in the literature to the sterility in F_1 of species crosses involving *N. sylvestris* as a male or female parent (cf. East and Hayes, *loc. cit.*, p. 28; Baur, 1911, p. 224). On the hybrids, mentioned above as being made in 1910 and growing in 1911, no "pure" seed was formed under bag so far as was determined at the time and indeed all maturing seed capsules that were protected fell long before the calyx had begun to wither or the ovary turn brown. Twenty or thirty shrunken but dried ripe seed capsules from unprotected flowers were collected from the plants of this H33 cross and its reciprocal (H34). These capsules were "cleaned" (Goodspeed, 1912, p. 129) carefully but no seed was found. The contents of one seed packet resulting from this "cleaning" was sown on the possibility that a few viable seeds might be present and have been overlooked, but no seedlings resulted.

A considerable amount of attention has been given this year to the phenomenon of sterility in the F_1 hybrid plants grown from the hybrid seed resulting from the 1911 crosses. The most

noticeable fact in this connection is the readiness with which the flowers fall from the pedicels at about the same time (peculiar to each hybrid) after anthesis. This situation seems to be connected with the formation of a definite abscis or separating layer within the individual pedicel at a distance approximately 1.5 mm. above its point of origin from the peduncle. This particular matter is at present under further investigation, but it appears to be true that this abscis layer is similar to that formed normally in connection with the fall of the leaf in deciduous trees, that it may in certain cases be formed some time before the flower bud opens, and in other cases begins its activity much later, and finally that it fails at times to form at all. The various F_1 hybrids exhibit considerable differences in connection with this particular point. A slight shaking of the main axis of one of the plants representing the cross H36 will cause practically all its flowers past anthesis to fall and also numbers of freshly opened flowers and even buds. The same is true, though in a less pronounced manner, of the plants grown from the seed of the reciprocal cross. In the case of the cross H38, on the other hand, the F_1 hybrids hold their flowers much more firmly and, though the great majority of the withering flowers finally fall, still when newly opened fully 50 per cent of the flowers will resist considerable strain before becoming separated from the pedicel. The situation in F_1 of the crosses H43 and H45 and their reciprocal is complicated in that very certain evidences of segregation are to be seen and various degrees of resistance to dropping of flowers seem at present to be correlated therewith. This will be further considered below. The flowers of the F_1 hybrid of the cross H40 fall almost as readily as do those on F_1 plants of the cross H36 mentioned above. The F_1 plants of H41 have not as yet come fully into flower (Dec. 10), but give evidence of possessing this peculiarity of loss of flowers to about the extent exhibited by the F_1 plants of H38. In all these hybrids it can be certainly stated that climatic variations, as they occur out in the field at any rate, are in no sense responsible for the dropping of flowers, nor can it be said that a period of rainy, cloudy weather will cause any very appreciable increase in this tendency (Goodspeed, *loc. cit.*, p. 141).

The first evidence that considerably less than absolute sterility can be claimed for certain of the F_1 hybrids from crosses of *N. sylvestris* with *N. Tabacum*-varieties was connected with the finding of a large number of entirely dry, brown, persisting seed capsules on the plants of cross H38. Six hundred and fifty of these capsules have been collected and more than an equal number remain on the 25 plants representing this cross. From a similar number of plants of the cross H36, 85 capsules have been taken. The plants grown in 1911 from the seed of the 1910 crosses have come up from their roots this year (1912) to make fairly vigorous plants and from one plant each of the cross and its reciprocal between 25 and 30 capsules have been taken. One hundred capsules of the 650 mentioned above have been found to contain nearly 900 perfectly formed seeds. A certain proportion of them undoubtedly are mere shells and contain no embryo (cf. East and Hayes, p. 30). One hundred of these seeds were arranged as noted in a previous report (Goodspeed, *loc. cit.*, p. 97) and germinated for five weeks under fairly well controlled conditions with the temperature approximately 21° C. At the end of this time 26 per cent had germinated, 90 per cent of which germination had taken place in 15 days with the first signs of germination noted at the end of three days. Five seedlings were allowed to develop and at the end of five weeks are normal in every respect and are considerably past the stage during which highest mortality of tobacco seedlings is usually expected. Four hundred of the remaining 550 seeds of this lot are being germinated under the same conditions and at the end of one week almost 25 per cent have germinated. The 1912 seed of H33 and its reciprocal have germinated to the extent of 17 per cent and 18 per cent respectively and a number of seedlings nearly four weeks old are developing normally. The brown, dry capsules examined were always much shrunken and withered. They were cut transversely and a brush was forced into the apparently empty spaces between the placentae and the wall of the matured ovary. When this operation is carried out over a rough, white surface under a strong light and the brush gently rubbed between the fingers any seeds that may have been taken up by the brush will fall on to the white surface and can be

detected. Of the 100 capsules (above mentioned) that were examined in this way 34 only contained no well-formed seed, while two contained 31 seeds each and a number over 20 seeds each. This seed was, as above stated, produced from unprotected flowers, but there is little reason to suppose that only those ovules matured which were fertilized by foreign pollen. The various hybrids differ among themselves as to the amount of pollen which an individual flower produces and in no case does it approach in amount that which is normal for the parental species. Sufficient is produced, however, so that in the case of 60 protected flowers examined just after the anthers were open the stigmas in 52 cases were covered with pollen. The crosses back upon the parents in which the F_1 hybrids are the male parents have been recently made and may give results, in connection with the amount of seed produced, which bear on this point. The hybrid inflorescences also are very dense and the flowers are produced in great profusion, so that in general the chances are greatly in favor of unprotected flowers being self- (close) pollinated or pollinated with pollen from other flowers on the same plant. The parental species are usually recognized as normally self-fertilized and no visible methods are present on the hybrids made between them to prevent self- and favor cross-pollination. Seed has not at this date matured under bag. In the case of the cross *N. Tabacum* var. *macrophylla* × *N. sylvestris* (H38) within each of over 30 bags containing from 30 to 50 flowers and buds an average of two maturing seed capsules can be seen through the paraffine bag to be persisting. In such cases the calyx is beginning to turn brown and the period during or before which the F_1 hybrid flowers generally fall has passed. In the case of the crosses H35 and its reciprocal H40 and H41, less than 20 maturing seed capsules, in a total of over 75 bags, seem to be persisting at this date. In general, however, it is plain that a small amount of viable seed is produced by hybrids of *N. sylvestris* with various *N. Tabacum*-varieties and there appears to be little doubt but that normal plants can be matured from the seedlings developed from such viable seed.

In connection with the seeding character exhibited by the above hybrids and especially by the F_1 plants of the cross *N.*

sylvestris $\times$ F_1 of the cross *N. Tabacum* "Maryland" $\times$ *N. Tabacum* "Cavala," it is interesting to note the partial report in the Gardner's Chronicle (vol. 50, 3d series, p. 309) of information, in connection with similar hybrids, given before the Fourth International Congress on Genetics by M. C. Bellair, Head Gardener at the National Palaces—"*N. sylvestris* pollinated by *T.* (sic!) *Tabacum* gives an F_1 generation exhibiting in many respects the characters of the latter species.* The F_2 germination yields a number of types of which some were fertile and some sterile. In F_3 plants resembling the two parents were obtained. When crossed with one another they produce a large series of forms—giants, dwarfs, white, rose, red colored and striped flowers." No mention is thus made of sterility in F_1. The *N. Tabacum* used in Mr. Bellair's cross is not made plain. In this connection it is to be noted that in certain F_1 plants produced from the seed of crosses H43, H44, and H45 a considerable quantity of what appears to be normally developing seed is forming. Though mature seed capsules at the present time are not obtainable, partially mature capsules borne on one plant of cross H45 exhibit from 30 to 50 seemingly *normally* developing ovules on the placentae. The flowers on this plant persist to a much larger extent than in the case of any of the other F_1 hybrids, no early browning of the placentae takes place, and in general this plant may be expected to yield a rather large quantity of seed, some of which should be viable. Of the 27 F_1 plants produced from the seed of this cross three or four almost exactly typical *N. Tabacum* "Maryland" individuals are present while the remaining 24 or 25 plants seem to exhibit a blending of the characters peculiar to the two Tabacum varieties involved in its pedigree. The plant refered to above which seems to be producing an unsual quantity of seed is of this blended type. The three F_1 plants nearly identical with the *N. Tabacum* "Maryland" parent also seem to be producing flowers which in certain laterals are persisting much longer than in the case of the other *N. Tabacum-varieties* $\times$ *N. sylvestris* hybrids and longer than the flowers in the case of the 23 or 24

* A similar degree of dominance of the *N. Tabacum*-varieties has been throughout observed in our cultures.

other "blends" of this same hybrid. Evidences of "segregation" are thus apparent in these F_1 plants and this segregation seems in certain cases to be correlated with increased seed production. With these facts in mind it seems possible that the *N. Tabacum* parent of Mr. Bellair's crosses was of hybrid origin and that F_1 individuals involving much the same cross have been produced in our cultures.

It seems reasonable, thus, to assume that the F_1 hybrids produced as a result of crossing *N. sylvestris* and *N. Tabacum*-varieties back and forth can be taken out of the absolutely sterile category and included among those hybrids that are only partially fertile (cf. Lock, 1909). With the reference to the cytological evidence on the question of the partial fertility of these hybrids a considerable investigation is under way, but the following facts previously noted seem pertinent in this connection. A separating or abscis layer is formed in the case of at least 50 per cent of the hybrid flowers before any fertilized ovules could be normally matured, and in the majority of cases where such a separating layer is not outwardly visible, a very small amount of viable seed is formed. Obviously the formation of this separating layer cuts off all possibilities of the transfer of food materials to the maturing ovules and it appears to be plain that in the parental species a very considerable demand upon available food supplies is made by such maturing seed (Goodspeed, 1913 (1)), Again it has been found that, given five or six flowers just past anthesis on the primary, terminal inflorescence of a lateral shoot, if the remaining buds, smaller leaves and partially opened flowers present on this lateral are allowed to remain and proceed to their full development, all or all but one of these five or six flowers past anthesis will fall in a week or ten days. On the other hand if all the terminal buds about these five or six flowers and all buds below in the axils of young leaves are removed, all or all but one of these five or six flowers past anthesis will ripen seed capsules which will contain on an average five viable seeds each. On the one hand, then, there definitely appear to be certain combined physiological and structural hindrances to any seed production at all in these F_1 hybrids and on the other hand cytological investigation may dis-

close other more fundamental difficulties in this connection that have to do with the lack of a normal maturing of the sex elements within the ovule, structural blocks to the proper penetration of the micropyle, etc. In any case, however, it seems at least conceivable—and the F_2 generation which it seems possible may be produced combined with cytological evidence will, it is hoped, help to settle this point—that such few viable seeds as are formed on these hybrids are produced apogomously or parthenogenetically (East, 1912, p. 131), on the basis of the fact that less than 1/100 of the number of viable seeds normal for the parental species are produced in those F_1 hybrids that mature any seed at all. Finally there must at least be borne in mind the suggestion which has also elsewhere been made (Goodspeed, 1913 (1), p. 179) in this connection that sexual reproduction vs. vegetative reproduction may be definitely inherited "physiological unit-characters" that behave alternatively in inheritance. As bearing on this later point it may be said that vegetative propagation by cuttings is comparatively easy in the case of all the hybrids discussed above just as it is in the case of *N. sylvestris* and in both cases this fact seems to be correlated with a biennial or perennial habit well known in the case of *N. sylvestris* but, to my knowledge, not previously noted to the same extent, at least, for *N. Tabacum*-varieties, the hybrids made between them or for hybrids between *N. Tabacum*-varieties and *N. sylvestris.* Thus, *Nicotiana sylvestris* under field conditions will reproduce itself "vegetatively" for at least three years. The 1910 *N. sylvestris* × *N. Tabacum* var. *macrophylla purpurea* crosses first grown in 1911 have similarly come up from their own roots this year (1912) and the 1911 crosses growing this year are forming adventitious roots wherever the lower materials are in contact with the soil.

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NOTES ON THE GERMINATION OF TOBACCO SEED

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BY
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[The relation between the age of tobacco seed and its viability and between the viability of parent and hybrid seed; together with notes on the value of treatment with sulfuric acid in hastening germination and increasing its amount.]

CONTENTS

PAGE

I. Introduction 199
II. Seed used 201
III. Methods 202
(*a*) Germinating conditions in general 202
(*b*) In connection with sulfuric acid treatment 203
IV. Results with sulfuric acid treatment 204
V. Age of seed vs. its germination 209
VI. The germination of parent vs. hybrid seed 215
VII. Summary of results 219
VIII. Discussion of results 220

I. INTRODUCTION

The difficulty experienced this year (1912) in germinating the seed of *Nicotiana attenuata* (U. C. B. G. 78/09) (Setchell, 1912, p. 24) was the occasion for a number of experiments with reference to the possibility of bringing about more rapid and complete germination in such cases. The interesting experiments of Love and Leighty (1912) in connection with the effect upon germination of sulfuric acid treatment in the case of clover

seed, cotton seed, etc., suggested the possibility that similar beneficial effects might be secured in the case of tobacco seed by similar treatment. As will be seen later on such has been found to be the case in that treatment with 70 per cent or 80 per cent sulfuric acid for lengths of time varying from five to fifteen or twenty minutes markedly increases the total amount and in many cases hastens the time of germination of the seed of a number of species of *Nicotiana*. In the preliminary experiments, seed of *N. attenuata* alone was used, but in order that the value of sulfuric acid treatment might be further investigated in the case of other species of *Nicotiana* it was first found to be necessary to make germination tests with untreated seed to determine the species which would give small total germination or slow germination under experimental conditions. This, in a sense, preliminary portion of the investigation has assumed rather large proportions and a considerable mass of data is at hand that has to do with the germination of seed of various ages derived from single plants of eight species and varieties of *Nicotiana* and also with the germination of hybrid seed obtained from crosses made between certain of these species and between certain of these varieties. Additional germination tests are at present under way which are designed to demonstrate the possible wider usefulness of sulfuric acid treatment in taking the place of other methods, peculiar to agricultural practice, of hastening the germination of tobacco seed and increasing its amount.

Detailed reports based upon definite germination tests in the case of pure lines of *Nicotiana* species are not, to the writer's knowledge, at present available. In general I think it has been assumed, and probably correctly, that tobacco seed will give rather high average total germination under field conditions and that little difficulty is experienced by commercial growers of tobacco in germinating the seed they use. The value of tobacco seed separation and the greater uniformity and vigor of the stand when only heavy seed, rather than mixed heavy and light seed, is sown has been pointed out by Shamel (1904) and others, while certain other more theoretical considerations in connection with the inheritance of certain characters in *Nicotiana* as correlated with the weight of the seed used to produce the plants

upon which they appear, have been in part dealt with in a previous communication (Goodspeed, 1912). Recently, also, it appears to have been shown that the hybrids made between *N. sylvestris* and various *N. Tabacum*-varieties may be considered to be partially fertile rather than completely sterile (Goodspeed, 1913), and this fact was brought to light both by a careful examination of the relatively few seed capsules (unprotected) that persist on these F_1 hybrids and the germination under controlled conditions of such little seed as was found within them. Again it is always conceivable that the so-called ratios which appear to be manifested in the results of breeding experiments from the Mendelian standpoint on the plant side may be modified by, or possibly are directly dependent upon, a high or low percentage of germination of the seed used to produce the plants the outward appearance of which is to be reported upon. In view of these several considerations, and especially that the more or less technical details connected with the breeding experiments reported upon in this volume may be entirely plain and that certain of the fundamental factors with which we are dealing in these experiments may be more thoroughly understood both by ourselves and by others, a rather detailed report upon the germination of the parent and hybrid tobacco seed used seems justified. It is hoped that further studies in this connection now being pursued may be reported upon in the near future.

II. SEED USED

As will be noted in the tables which follow, the seed used in the germination tests herein reported upon was taken from single plants representing the following species and varieties of *Nicotiana* and various crosses made between them. The designations given refer to the acquisition number, the year first sown and the hybrid number in the U. C. B. G.

110/05—*N. Tabacum* var. *calycina* (Setchell 1912, p. 6).
22/07—*N. Tabacum* var. *macrophylla* (*ibid.*, p. 8).
78/05—*N. Tabacum* "Maryland" (*ibid.*, p. 5).
78/09—*N. attenuata* (*ibid.*, p. 24).
53/03—*N. acuminata* variety (*ibid.*, p. 23, and Goodspeed, 1912, pp. 119–122).

192/08—*N. acuminata parviflora* (Goodspeed, *loc. cit.*)
150/07—*N. acuminata grandiflora* (*ibid.*)
22/02—*N. Langsdorfii* (Setchell, 1912, p. 15).
107/01—*N. sylvestris* (*ibid.*, p. 29).
H18—110/05 × 78/05.
H20—78/05 × 110/05.

The germination of the seed obtained in different years from single plants of the above species and varieties of *Nicotiana* will be dealt with in the following pages with reference first to the effect of sulfuric acid treatment upon germination of seed of particular strains and seed of various ages, second to the relation between the age of tobacco seed and its germination and lastly with reference to the germination of parent versus hybrid seed. The technique involved in the obtaining of "pure" seed under bag and in performing cross pollinations has been described elsewhere (Goodspeed, 1912, p. 126–129) as also the methods employed in cleaning the seed, sowing it, etc. Pure seed from a single plant only has been employed in the germination tests in the greater majority of cases and unless otherwise stated. In practically every instance also and unless similarly noted the seed tested was a part of that used on a given year to produce the plants grown in the U. C. B. G. and thus is definitely related to the various breeding experiments therein being carried on. In general the seed had been stored in the laboratory in boxes and similarly wrapped (Goodspeed, *loc. cit.*) and thus any condition that might have increased deterioration in viability acted upon all the seed in the same way.

III. METHODS

(*a*) Germinating Conditions in General

Entirely satisfactory conditions for germination were not available. The seeds were counted out on to containers of the type elsewhere described (Goodspeed, *loc. cit.*, p. 96) except that in most cases finger bowls rather than drinking glasses were used and thus larger surfaces were secured to hold the two hundred seeds that were tested in the case of each plant. The seeds were carefully arranged in rows in groups of one hundred seeds each. This arrangement made it possible to check rapidly the

results of each observation. A circle drawn in lead pencil about one group of one hundred seeds distinguished it in the matter of keeping the records of duplicate tests. In every case there was no preference given to the larger, better formed seeds in arranging the seeds to be tested. On the contrary the relatively few ill-formed seeds were included as they occurred, since one of the primary objects of the investigation was concerned with determining what proportion of viable seeds each plant normally produces. The criterion of germination was the same as that elsewhere noted (Goodspeed, *loc. cit.*, p. 98). A number of tests demonstrated the fact that 96.8 per cent of the seeds thus decided upon as showing germination will give rise to vigorous seedlings the development of which has been watched for two weeks after germination. It seems certain that this percentage would be higher if it were possible to eliminate the injurious effects of handling the very small germinating seeds, which are difficult to pick up lightly.

An even approximately standard germinating case was not available. The finger bowls covered by the seed holders were placed on sand in a shallow glass-covered case built on the top of a greenhouse bench. Unfortunately strictly constant temperature conditions were not possible in this germination test except toward the end of the experiments herein reported upon. At the start of the experiments the temperature of the water in the finger bowls varied between 27° C for the average day temperatures and 22° C for the night temperatures (cf. Garman, 1910). This amount of variation was somewhat cut down after the end of two weeks and during the last month of the experiments the temperature conditions under which germination took place were very fairly constant at 25° C.

(*b*) In Connection with Sulfuric Acid Treatment

The work herein reported on that is connected with the effects of treatment with sulfuric acid upon the germination of tobacco seed is presented merely as preliminary with regard to more detailed experiments which are at present in progress. The value of sulfuric acid treatment has been established, however, and this fact seems to warrant the inclusion of such experimental evidence as is at hand as well as the methods employed

in this connection. In general the suggestions given by Love and Leighty (1912) have been followed in modified form. The action of concentrated sulfuric acid (sp. gr. 1.84) for lengths of time as short as one minute in the case of 53/03 ate the seed coats entirely away and destroyed any possibilities of germination. This was proved by a number of tests and by microscopical examination. It is, however, entirely conceivable that the seed of other strains of tobacco, better protected as to seed coats, will give more favorable results with the concentrated acid—i.e., seed of U. C. B. G. 150/07 possibly. A considerable number of experiments showed that treatment with 50 per cent, 70 per cent, and 80 per cent sulfuric acid gave the best results, and the majority of the tests were made after treatment with 80 per cent acid for varying lengths of time.

A small quantity of the seed to be tested was placed in a small vial and covered with the 80 per cent acid at room temperature. After allowing the acid to act for the desired length of time the vial was at once filled with tap water and the seed and greatly diluted acid poured through strong cloth which served to retain the small seeds. The seeds were washed for one or two minutes on the cloth with a fine stream of tap water and then transferred to another cloth-covered vial in which they were further washed with running water. The effects of prolonged washing after sulfuric acid treatment will be further discussed below. After the washing the seed was counted out in two lots of one hundred each, as above noted, on to the blotting paper holders and placed in the germinating case in the greenhouse.

IV. RESULTS WITH SULFURIC ACID TREATMENT

The following table expresses the effect of sulfuric acid treatment—50 per cent, 70 per cent, and 80 per cent acid—upon the germination of the seed of 78/09 produced in 1911. Because of the small amount of seed available duplicate tests were not possible and only fifty seeds were used in each individual test. After the treatment with acid the seeds were washed with running water for an hour while in the control the seeds were placed dry on the filter paper.

TABLE 1

Year	Plant Designation	Treatment	Number of seeds that germinated on the days indicated																			Per cent germinated
			5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	40	
1911	78 09	Control		4		1		9		1	1											32
1911	78/09	50% sulfuric acid for 15 minutes		2	4			7		1												28
1911	78/09	50% sulfuric acid for 30 minutes		1	5			6		9		2										46
1911	78/09	50% sulfuric acid for 60 minutes			3			6														18
1911	78/09	70% sulfuric acid for 10 minutes		1	2			9		6		3			3							48
1911	78/09	70% sulfuric acid for 15 minutes		1	1			13	2		2		3									44
1911	78/09	70% sulfuric acid for 20 minutes			1			8		3		3					2					34
1911	78/09	80% sulfuric acid for 5 minutes		1	8				3			10	4		1							54
1911	78/09	80% sulfuric acid for 10 minutes		1	3			14			7	3			2	1						62
1911	78/09	80% sulfuric acid for 15 minutes						1		2	1				1							10

From the above figures it is evident that treatment with 80 per cent sulfuric acid for lengths of time not much greater than ten minutes will materially increase the amount of total germination in the case of the seeds of *N. attenuata*. More rapid germination of the treated seed is not, however, in general apparent, for at the end of fifteen days in only two instances —70 per cent for fifteen minutes and 80 per cent for ten minutes —was the germination of the treated seed greater than in the control. The optimum lengths of treatment in this case evidently lie between thirty and sixty minutes for the 50 per cent acid, between ten and fifteen minutes for the 70 per cent, and probably not much beyond ten minutes in the case of the 80 per cent acid. A considerable number of other tests with seed of 78/09 of 1911, using both stronger and weaker grades of sulfuric acid for various lengths of time, were carried through. The above table, however, includes the most significant results, though it deals by no means with only those tests in which favorable results after sulfuric acid treatment were obtained.

Seeds of a number of other *Nicotiana* species which showed relatively low germination (see page 210) were treated with sulfuric acid in an effort to increase germination and a number of the results are shown in tabulated form below. In each case except the last the seeds were washed after the acid treatment for from forty-five minutes to one hour in running water.

The prefixes attached to the seed numbers designate the year upon which the seed was gathered. Thus 1906 53/03 refers to seed of 53/03 gathered in 1906 or six years old at the time of making the tests.

As will be seen in the table below, the effect upon germination of treatment with 70 per cent and 80 per cent sulfuric acid has been investigated in the case of old seed of *N. Tabacum* "Maryland" and old and new seed of two *N. acuminata*-varieties. The relative amount and rapidity of germination in the old vs. new untreated tobacco seed is taken up at greater length elsewhere (see page 210). In every case in the table below treatment with diluted sulfuric acid has resulted in a rather striking increase in total germination as compared with the controls and also in a rather marked increase in rapidity of germination in the case of the treated seed.

TABLE 2

Year	Plant Designation	Treatment	Number of seeds that germinated on the days indicated																		% germinated averaged duplicate tests
			5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	40	
1906	53/03	Control	.....	1	.....	.....	37	8	18	2	.....	.....	1	.....	2	.....	.....	6	.....	.....	.....	
			.....	.....	2	.....	21	18	26	3	.....	.....	3	.....	4	.....	.....	1	.....	.....	.....	51.0*
1906	53/03	80% sulfuric acid for 5 minutes	2	4	7	16	16	3	1	.....	.....	1	5	29	5	7	.....	.....	.....	.....	.....	
			.....	5	6	21	20	.....	1	.....	.....	1	3	22	8	5	1	.....	.....	.....	.....	63.0*
1907	53/03	Control	.....	.....	.....	1	.....	9	.....	.....	.....	.....	.....	4	9	.....	6	3	.....	.....	.....	
			.....	.....	.....	.....	3	8	.....	4	.....	.....	.....	6	11	1	9	2	.....	.....	.....	38.0
1907	53/03	70% sulfuric acid for 10 minutes	2	.....	1	.....	.....	.....	2	1	27	15	.....	6	4	1	3	.....	.....	.....	.....	
			3	1	1	.....	.....	.....	6	2	19	17	.....	2	5	1	1	2	.....	.....	.....	61.0
1907	78/05	Control	.....	.....	6	60	4	5	.....	2	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
			.....	.....	3	42	12	9	2	5	.....	1	2	.....	.....	.....	.....	.....	.....	.....	.....	77.5
1907	78/05	80% sulfuric acid for 5 minutes	.....	17	43	8	2	3	5	1	2	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	
			.....	30	36	11	1	2	1	.....	2	.....	1	1	.....	1	.....	.....	.....	.....	.....	84.0
1908	150/07	Control	.....	.....	.....	5	2	5	3	.....	2	2	1	1	.....	.....	.....	.....	.....	.....	.....	
			.....	.....	.....	6	3	.....	.....	.....	2	1	4	2	.....	.....	.....	.....	.....	.....	.....	19.5
1908	150/07	80% sulfuric acid for 5 minutes	.....	.....	.....	2	11	12	3	9	3	3	3	3	.....	.....	.....	.....	.....	.....	.....	
			.....	.....	.....	3	15	21	4	5	2	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	50.0
1912	150/07	Control	3	.....	2	2	1	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
			5	.....	2	1		2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	9.5
1912	150/07	80% sulfuric acid for 5 minutes—washed 45 minutes	32	11	3	2	5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	(53)
		80% sulfuric acid for 5 minutes—washed 20 hours	14	11	3	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	(29)41.0

*150 seeds in each test

The extremely low percentage of total germination in the case of 1912 150/07 is rather surprising, but in the table on page 212 it will be seen that the 1912 seeds of 53/03 and 192/08—the other two *N. acuminata*-varieties cultivated in the U. C. B. G.—also showed a remarkably low per cent of total germination. In general no difficulty has been experienced in past years in securing a fairly high per cent of germination in the case of *N. acuminata*-varieties under the germinating conditions elsewhere described (Goodspeed, 1912, pages 98 and 132), although under such conditions germination in *N. acuminata* has always been slow as compared with practically all the other *Nicotiana* species grown. In the case of 1912 53/03, 192/08 and 150/07 the seed was taken from the ripe and opened capsules on the plants and did not lie in the seed packets for four or five months as is the rule where the seed is sown in March or April following the gathering of the seed in November or December. This period of "after ripening" or further drying of the seed of *N. acuminata*-varieties may be essential for a high percentage of germination of their seed under experimental conditions. It is also entirely possible that conditions for germination in the case of the seed of *N. acuminata*-varieties may be more favorable when the seed is sown in soil and placed in an unheated propagating house in which the variation in daily temperature covers a wide range. In this connection the striking increase in germination when this 1912 150/07 seed is treated with 80 per cent sulfuric acid for five minutes and the acid washed off for forty-five minutes in running water is unusually significant. The effect of prolonged washing after treatment with sulfuric acid is brought out in the above table (table 2). As will be seen there in the case of 1912 150/07 when washed in running water for forty-five minutes one of the duplicate tests showed 53 per cent germination, while the other similarly treated but washed for twenty hours gave only 29 per cent germination. A similar injurious effect of prolonged washing after sulfuric acid treatment has been noted by Love and Leighty (*loc. cit.*, p. 314). Additional tests further taking up this particular point are in progress.

It seems thus possible on the basis of these relatively few preliminary tests to state that a marked benefit is to be derived

from the treatment of tobacco seed with 70 per cent or 80 per cent sulfuric acid for varying lengths of time in hastening germination and particularly in increasing its amount. It is hoped that the results of further experiments in this connection may be reported upon in the near future.

V. THE RELATION BETWEEN THE AGE OF SEED AND ITS VIABILITY

The following tables deal with the results of some thirty-five germination tests carried through in the effort to determine in a preliminary fashion the relation between the age of tobacco seed and its viability. As will be seen in only one case—i.e. 110/05—was it possible to test any one species as to the viability of the seed of consecutive years from the year first grown in the U. C. B. G. to the seed of the present year. In other cases—i.e., 22/07—the tests represent seed of an uninterrupted series of years but the seed of the first year or so as grown in the U. C. B. G. is missing. In still other cases—i.e., 53/03—tests are lacking for the oldest seed and the series is also interrupted.

Table 3 below represents a general compilation by years of the results of the various tests. Apart from the remarkably high percentage of germination among the six, seven, and eight-year-old seed the most interesting points brought out in table 3 have to do with the germination of the *N. acuminata*-varieties in general, the low percentage of germination of 22/02 more than two years old, and the low germination percentages in many cases for the seed gathered in 1910.

With reference to this last point no explanation can be offered except that during the season 1910 conditions for maximum growth and development were not so distinctly favorable as in the preceding year or in the two following years. Actual climatic conditions do not seem to have differed greatly from the average, but the garden records show that the parent strains of tobacco—*N. Tabacum*-varieties in particular—did not germinate as rapidly or as fully in 1911 as normally. A point of this sort in connection with the propagation of material connected with hybridization experiments on the plant side may in general

TABLE 3

Year	Plant Designation	Number of seeds that germinated on the days indicated																			Per cent germinated averageduplicate tests
		5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	40	
1905	110/05		13	4	44	3		1	3	3											71.0
			18	2	39	4		2	5	1											
1906	110/05		36	4	15	1	5														59.0
			29	7	18		1	2													
1906	53/03		1			37	8	18	2			1		2			6				51.0*
				2		21	18	26	3			3		4			1				
1906	78/05				1	1		2						19		1	2				25.5
				1	2		1	1					3	9	4	2	1	1			
1906	22/02																				0.0
1907	110/05		55	5	7	1	1														70.5
			58	3	9	1			1												
1907	53/03				1		9						4	9		6	3				38.0
						3	8		4				6	11	1	9	2				
1907	78/05			6	60	4	5		2	2											77.5
				3	42	12	9	2	5		1	2									
1907	22/02			1	1						1										3.0
					1						2										
1908	110/05		44	11	13	4	2	1													74.5
			47	6	13	4	1	1	1						1						
1908	53/03	2			3		1		7	18	6		13		2		1		1		57.0
		1	1		5				3	21	7		12	5	1		2		2		
1908	22/02		1																		0.5
1908	22/07	8	50	15	10	2															88.0
		11	34	28	13	2	1					2									

*150 seeds in each test

TABLE 3—(*Continued*)

Year	Plant Designation	Number of seeds that germinated on the days indicated																		Per cent germinated average duplicate tests
		5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	40	
1908	150/07	....	....	.....	5	2	5	3	....	2	2	1	1	....	....	....	....	....	....	....	19.5
		...	.....	.....	6	3	....	....	....	2	1	4	....	2	....	....	....	.....	....	....	
1909	110/05	....	46	11	19	.....	2	2	1	....	.....	.....	....	....	.....	....	....	.....	.....	.....	79.5
		..	40	14	22	....	1	.....	1	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
1909	78/05	.....	....	.....	68	14	13	1	1	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	71.5*
		....	....	.....	.....	.....	1	8	5	12	3	5	.....	3	....	3	2	2	2	....	
1909	22/07	.....	3	60	24	3	2	2	....	1	....	1	.....	....	....	.....	.....	.....	.....	.....	96.0
		....	41	26	23	3	.....	2	....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	
1909	22/02	.....	....	9	10	8	4	2	1	.....	1	1	1	.....	....	.....	....	1	....	....	39.0
		....	...	5	12	9	3	5	2	....	1	....	2	...	.....	.....	.....	1	....	....	
1910	110/05	...	....	8	11	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	26.5
		....	....	15	15	4	....	....	....	.....	..	.....	.....	.....	.....	.....	...	...	.....	.....	
1910	53/03	....	....	8	5	2	....	3	10	....	2	1	1	....	8	6	6	....	2	....	58.0
		....	...	6	4	4	4	1	5	1	3	2	6	....	4	10	5	3	4	....	
1910	78/05	....	5	14	25	1	1	7	1	2	2	2	....	2	1	.....	....	1	1	....	62.5
		....	3	18	27	....	.....	5	1	1	3	.....	....	1	....	....	1	....	...	.....	
1910	22/07	....	53	24	12	....	1	....	....	...	....	.....	..	....	....	.....	.....	.....	..	.....	89.5
		16	66	2	3	....	1	....	.....	1	...	.....	..	....	.....	.....	.....	.....	.....	.....	
1910	107/01	.....	.....	.....	3	24	29	28	12	....	....	.....	.....	.....	.....	.....	.....	.....	..	.....	95.0
		.....	...	....	2	20	36	23	13	.....	....	.....	.....	.....	.....	.....	.....	..	.....	.....	
1911	110/05	.....	66	17	2	1	.....	1	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	84.0
		....	62	15	.....	4	.....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
1911	53/03	....	.....	..	1	1	.....	.....	.....	.....	.....	.....	14	....	5	....	1	2	....	....	25.0
		....	.....	3	2	1	...	.....	.....	.....	.....	1	11	....	6	....	1	1	....	....	
1911	78/05	....	7	29	58	2	....	....	....	.....	.....	.....	...	....	....	.....	.....	.....	.....	.....	95.0
		....	10	30	42	12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	

*cf page 221

TABLE 3—(*Continued*)

Year	Plant Designation	No of seeds that germinated on the days indicated																			Per cent germinated average duplicate tests
		5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	
1911	22/02		15	10	17	22	7	3	1							1					
			12	19	5	27	16														77.5
1911	22/07		97	1																	
		13	72	11	1				1												98.0
1912	110/05	22	59	2	1		2		1		1										
		23	50	9	6		1														88.5
1912	53/03		1	3					1			1									
				3	4	1															7.0
*1912	78/05		32	50	12	3	2														
			32	42	17	3	2	2													98.5
1912	22/07	49	31	13	3	1															
		53	30	9	1	2															96.0
1912	192/08	1	1	1																	
					1							1									2.5
1912	150/07	3		2	2	1	1														
		5		2	1		2														9.5
1912	107/01							7	1		68	23	1								
								8	2		57	30	1	2							100.0

*From capsules still green

be noted on a given year, but its full significance is lost unless a little of the seed is germinated each year under controlled conditions and in a manner which makes possible more or less exact germination counts. In this connection it may be said that the possession of such records would have been greatly appreciated in connection with the results of certain experiments reported on in a previous communication (Goodspeed, 1912, pages 87–117). The general low germination of the seed gathered in 1910 is further brought out in table 4 below. This table is a different expression of the results set down in table 3. It gives the average per cent of germination for each of the eight years in the case of *all* the seed of each year germinated and thus is not an accurate expression of the relative amounts of germination in each case. In the case of 1912 tests the results of the tests with the *N. acuminata*-varieties—53/03, 192/08, and 150/07—have not been included, though appearing in table 3, since their extremely low percentage of germination seems to show that other factors have entered in to affect the results which are thus not typical. The low germination of the seed of 1910 is again seen in table 5, which is self-explanatory.

These tables show the rather surprising viability of relatively old tobacco seed (cf. Hayes, 1912, p. 3). The percent of germination in the case of eight-year-old seed of 110/05 or five-year-old seed of 22/07 makes it seem probable that twelve and possibly fifteen-year-old seed of *N. Tabacum*-varieties would germinate to a certain extent at least—i.e., that deterioration as to viability is a gradual process. The germination of the seed of 22/02, on the other hand, makes it seem at least possible that there is a more or less sharp end point as to the age limit of viability and that after being kept a year or two more the seed of 1905 of 110/05 may fail to show any germination. As will be seen in table 3 the 1909 seed of 22/02 germinated to the extent of 39 per cent, while among the seed of the same species one year older only one seed in 200 germinated and only six seeds germinated among 200 that were two years older. There is then, in the case of 22/02, a sharp drop in viability from nearly 40 per cent to not over 5 per cent in one year as the seed is kept beyond four years. At the end of the tests this older seed of 22/02 was

TABLE 4

Year	Plant designation	% germinated
1905	110/05	71.0
1906	110/05, 53/03, 78/05, 22/02	33.8
1907	110/05, 53/03, 78/05, 22/02	46.5
1908	110/05, 53/05, 22/02, 22/07, 150/07	47.7
1909	110/05, 78/05, 22/02, 22/07	71.2
1910	110/05, 53/03, 78/05, 22/07, 107/01	66.7
1911	110/05, 53/03, 78/05, 22/02, 22/07	75.8
1912	110/05, 78/05, 192/08, 107/01	95.7

TABLE 5

Year	Plant designated	% germinated
1905	110/05	71.0
1906	110/05	59.0
1907	110/05	70.5
1908	110/05	74.5
1909	110/05	79.5
1910	110/05	26.5
1911	110/05	84.0
1912	110/05	88.5
1908	22/07	88.0
1909	22/07	96.0
1910	22/07	89.5
1911	22/07	98.0
1912	22/07	96.0

found to be functionless and decaying. The relatively low percentage of germination in the case of old and also this year's seed of *N. acuminata*-varieties is interesting in view of certain experiments with this species previously reported upon (Goodspeed, 1912), which are still being carried on. Table 6 is again a condensation of table 3 with reference to the three *N. acuminata*-varieties.

In general it appears that the older seed of *N. acuminata*-varieties gives the highest percentage of germination throughout. Why this should appear to be true is not plain, but undoubtedly the conditions—i.e., temperature conditions—under which the tests were carried out cannot be the most favorable for the germination of the seed of this particular species. The same fact may, of course, have influenced the results of the germination tests

TABLE 6

Year	Plant designated	% germinated
1906	53/03	51.0
1907	53/03	38.0
1908	53/03	57.0
1910	53/03	58.0
1911	53/03	25.0
1912	53/03	7.0
1908	150/07	19.5
1912	150/07	9.5
1912	192/08	2.5

in the case of other species also, but in general the conditions under which germination took place seemed to be the most generally favorable that were available.

VI. THE GERMINATION OF HYBRID VS. PARENT SEED

On the possibility that there might be some correlation between the amount and time of germination and such segregation as was observed in certain of the hybrids produced in the U. C. B. G. a series of germination tests was carried out with hybrid seed produced by the hybrids made between a number of *N. Tabacum*-varieties. The following table gives the germination of the seed of 110/05, the germination of seed of 78/05 and the germination of the seed resulting from the cross 110/05 × 78/05 and its reciprocal, together with the germination of the seed produced by one F_1 hybrid plant of both cross and reciprocal and the seed of three F_2 plants produced from the seed of these last two individuals. H18 represents the cross 110/05 × 78/05; H20 its reciprocal.

As will be seen the amount of germination of the seed of the cross, the seed of the F_1 hybrid and of the various F_2 hybrids was very nearly identical in each of the two cases—i.e., 1909 H20, 1910 F_1 H20 P26, 1911 F_2 H20 P26 P11 and P26 P25 all show practically the same amount of germination and the germination was scattered over some nineteen to twenty-five days—while in 1909 H18, 1910 F_1 H18 P49, 1911 F_2 H18 P49 P25, P49 P24 and P49 P22 very heavy germination took place within a week. In the various generations of cross H18 the amounts of

TABLE 7

Year	Plant Designation	Number of seeds that germinated on the days indicated																		Per cent germinated average duplicate tests
		5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	40	
1905	110/05		13	4	44	3		1	3	3											
			18	2	39	4		2	5	1											71.0
1906	110/05		36	4	15	1	5														
			29	7	18		1	2													59.0
1907	110/05		55	5	7	1	1														
			58	3	9	1			1												70.5
1908	110/05		44	11	13	4	2	1													
			47	6	13	4	1	1	1						1						74.5
1909	110/05		46	11	19		2	2	1												
			40	14	22		1		1												79.5
1910	110/05			8	11																
				15	15	4	1														26.5
1911	110/05		66	17	2	1		1													
			62	15		4															84.0
1912	110/05	22	59	2	1		2		1		1										
		23	50	9	6		1														88.5
1909	H20		1	16	64	8	4	2	2				1								
			2	22	55	8	4	2	2												96.5
1910	F_1 H20		32	56	5	4															
	P26		34	63	2	1															98.5
1911	F_2 H20	3	12	15	30	16	5			4		2									
	P26 P11	3	17	10	45	15		1		5											91.5
1911	F_2 H20	19	69	4	2	1															
	P26 P25	22	63	2	2	1	4		1												95.0
1909	H18	3	73	17							1										
		2	69	12	1	1															89.5

TABLE 7—*(Continued)*

Year	Plant Designation	Number of seeds that germinated on the days indicated																		Per cent germinated average duplicate tests
		5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	40	
1910	F_1 H18	86	11	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
	P49	80	17	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	97.5
1911	F_2 H18	31	63	4	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
	P49 P25	26	62	11	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	99.0
1911	F_2 H18	39	59	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
	P49 P24	26	72	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	99.0
1911	F_2 H18	6	80	8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
	P49 P22	6	79	8	2	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	95.0
1906	78/05	.....	.....	.....	1	1	.....	2	.....	.....	.....	.....	.....	19	.....	1	2	.....	.....	.....	
		.....	.....	1	2	.....	1	1	.....	.....	.....	.....	3	9	4	2	1	1	.....	.....	25.5
1907	78/05	.....	.....	6	60	4	5	.....	2	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
		.....	.....	3	42	12	9	2	5	.....	1	.....	.....	2	.....	.....	.....	.....	.....	.....	77.5
1909	78/05	.....	.....	.....	68	14	13	1	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
		.....	.....	.....	.....	.....	1	8	5	12	3	5	.....	3	.....	3	2	2	2	.....	71.5
1910	78/05	.....	5	14	25	1	1	7	1	2	2	2	.....	2	.....	.....	.....	1	1	.....	
		.....	3	18	27	.....	.....	5	1	1	3	.....	.....	1	1	.....	1	.....	.....	.....	62.5
1911	78/05	.....	7	29	58	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
		.....	10	30	42	12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	95.0
1912	78/05	.....	32	50	15	3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
		.....	32	42	17	3	5	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	98.5

total germination are nearly identical except in the case of the seed resulting from the cross—i.e., the seed of 1909 H18.

In general, then, the most significant facts brought out by these last tests have to do with the exceedingly high percentage of germination among the two sets of hybrid seed from one to three years old and the more or less scattering germination of the seed of H20 throughout. In connection with this latter situation the seed of 78/05, as will be seen, shows a remarkably long period during which germination takes place—i.e., over four weeks except in the case of this year's seed and that of 1911. There seems thus to be some difference between the cross and its reciprocal as to the rapidity of germination and the relatively slow germination of 78/05 is seen only in that one of the two hybrids in which this *N. Tabacum*-variety is the female parent. On the other hand the germination of H20 is practically identical, in the length of the period during which germination takes place, with that of 110/05 in general, while the germination of H18 is much more rapid throughout than either 110/05 or 78/05. The significance of these facts is not entirely clear. The increase in amount of total germination in the case of all the hybrid seed as compared with the seed of the parental varieties during the various years is rather striking. In connection with the seed produced by the F_1 plants of H18 and H20 the increased germination of the hybrid over the parent seed of the same year might be referred to the general stimulating effect of heterozygosis (East and Hayes, 1912). In this connection it may be noted that the plants which bore this F_1 hybrid seed showed in a general way an increase in vegetative characters as compared with the parental varieties, but to no such extent as in the F_1 hybrids involving *N. Tabacum*-varieties and *N. sylvestris* as parents (cf. Goodspeed, 1913). The fact, however, that these latter hybrids were found to produce very little seed, only part of which would germinate seems to indicate that increase of vegetative characters due to heterozygosis, is not necessarily correlated with the production by such hybrids of abnormally large or even normal amounts of seed or of seed which will show a high percentage of total germination.

TABLE 8

Year	Plant designation	% germinated
1909	110/05	79.5
1909	H20	96.5
1909	H18	89.5
1909	78/05	71.5
1910	110/05	26.5
1910	F_1 H20 P26	98.5
1910	F_1 H18 P 49	97.5
1910	78/05	62.5
1911	110/05	84.0
1911	F_2 H20 P26 P11	91.5
1911	F_2 H20 P26 P25	95.0
1911	F_2 H18 P49 P25	99.0
1911	F_2 H18 P49 P24	99.0
1911	F_2 H18 P49 P22	95.0
1911	78/05	95.0

Table 8 brings out more clearly the relative amounts of germination of hybrid and parent seed on corresponding years.

In the case of two and three year-old seed the higher percentage of germination for the hybrid seed as compared with the seed of the parental *N. Tabacum*-varieties is marked, while in one-year-old seed which involves the seed of F_2 hybrids the contrast is much less striking. Further tests are contemplated which may throw some light on the significance of such results as these.

VII. SUMMARY OF RESULTS

The following summary of results is based upon the above description of germination tests and others involving in all over 20,000 seeds produced from single plants of ten species and varieties of *Nicotiana* and from single plants representing the original hybrid seed and the F_1 and F_2 generations in the case of two hybrids made between two *N. Tabacum*-varieties.

(1) The action of 80 per cent sulfuric acid upon tobacco seed for lengths of time not over ten to twelve minutes increases markedly the total amount of germination and in certain cases increases rapidity of germination.

(2) The action of concentrated sulfuric acid (sp. gr. 1.84) for periods of time as short as one minute killed the seed used.

(3) A markedly injurious effect of prolonged washing with running water after sulfuric acid treatment was noted.

(4) Six, seven, and eight-year-old tobacco seed was found to give relatively high percentage of total germination in most cases.

(5) Rapidity of germination in general was found to be independent of the age of the seed and to be characteristic of the seed of certain species or varieties throughout and not characteristic of others.

(6) A certain period of "after ripening" seemed to be essential for average germination percentages as shown in the case of 1912 *N. acuminata*-varieties. Seed taken from dehiscing capsules on the plants in the field gave very low percentages of germination in the case of *N. acuminata*-varieties alone.

(7) F_1 hybrid seed three years old gave higher percentages of germination than the seed of the parents of the corresponding cross and of the same age.

(8) The relation between the germination of parent and hybrid seed indicate that interesting and possibly important supplementary results can be obtained by the germination under controlled conditions of the seed used in hybridization experiments.

VIII. DISCUSSION OF RESULTS

The preliminary nature of this communication makes unnecessary any detailed discussion of the results obtained. Throughout mention has been made of further germination tests at present in progress or contemplated which are designed to investigate further certain of the more interesting points merely touched upon in the present paper.

The real significance of the action of sulfuric acid in hastening or increasing germination opens a field for investigation which should prove important, especially if such treatment is to be included in general agricultural practice. Results at present at hand seem to leave no doubt that in the case of *N. acuminata*-varieties at least the action of sulfuric acid is not restricted to weakening the hard outer coverings of the seeds so that water can penetrate more rapidly or that the germinating seed can break them more easily, but that its action is further strikingly effective in increasing the rate of growth during at least the first three months of the plant's life.

Further lines of investigation contemplated have to do with the germination of seed taken from capsules still green (see table 3) and taken at various stages during the maturing of the seed capsules on the plant and the relation between the vigor of the plant and the percentage of germination of the seed it produces. In this latter connection it will noted that in table 3 in the case of 1909 78/05 the first of the duplicate tests germinated to the extent of 97 per cent, while the second showed only 46 per cent germination. In this case only the seed used was taken from two plants of *N. Tabacum* "Maryland" and not from one individual alone. The seed in the first of the duplicate tests which gave the high percentage of germination was produced by a tall, much-branched plant of 1909 78/05, while that used in the second test which gave the low percentage of germination was taken from a plant of the same year which was relatively inferior in general vegetative characters.

I am glad to acknowledge my indebtedness to Professor W. A. Setchell for his interest in and suggestions concerning the experiments reported upon above, and to Mr. W. G. Perrine, upon whose assistance and coöperation the success of much of the work has depended.

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QUANTITATIVE STUDIES OF INHERITANCE IN NICOTIANA HYBRIDS. III.

BY
THOMAS HARPER GOODSPEED

Two previous reports have partially outlined the results obtained in an effort to contribute to the knowledge of the inheritance of quantitative characters (Goodspeed, 1912 and 1913). Crosses involving three flower-size varieties of *Nicotiana acuminata* (Grah.) Hook. have been investigated up to and including F_3. The first and second generations of these hybrids have already received consideration and it is the purpose of the present paper to review the results previously obtained and to take up briefly the nature of the F_3.

The tables which follow are based upon over 13,000 measurements of spread and length of corollas of flowers borne upon the three flower-size varieties of *N. acuminata* and on the hybrids made between them. The method of designation of parents and hybrids is the same as that employed in a previous communication (1912). The parents, one F_1 and one F_2 population, and the F_3 were grown in the summer of 1913 under conditions as nearly as possible identical with those under which the plants in this experiment were grown in 1912.

The arrangement of the material in the form of frequency distributions and the consequent rearrangement of the data, and in some cases the combination of two or more groups of hybrids of the same pedigree, give to the figures an appearance somewhat different from that presented in the earlier reports upon the same hybrids. The frequency distributions for corolla spread of *individual plants* formed the main basis for these earlier reports. In the present connection such distributions are not of sufficient interest to warrant their inclusion. The statistical constants for the tables which follow are not given, as their significance is doubtful with such small populations as were measured.

During this past year the length of the corolla as well as the spread of the corolla, which alone was considered in previous measurements, has been investigated. The term "length of corolla" refers to the distance from the point of insertion of the corolla upon the receptacle to the top of the corolla tube.

The measurement of corolla length as taken in the field was the distance from the base of the calyx tube to the point where the flattened corolla limb bends more or less sharply to join the corolla tube. The measurement of "corolla diameter" or "spread" was taken during the past two years in the same manner as described in another report for the earlier measurements (1912).

TABLE 1

Variety I, Variety II and Hybrids—Spread

Designation	Class centers in mm. 20	21	22	23	24	25	26	27	28	29	30	31	32	33	No. of plants	Mean flower-size
Variety I																
1910	....	....	....	....	....	....	....	6	....	....	....	....	....	....	6	26.93
1911	....	....	....	....	....	....	....	5	....	....	....	....	....	....	5	27.20
1912	....	....	....	....	....	....	....	....	....	....	....	....	1	4	5	32.73
1913	....	....	....	....	....	....	....	....	1	3	2	2	....	....	8	30.10
Variety II																
1910	5	1	....	....	....	....	....	....	....	....	....	....	....	....	6	20.29
1911	7	....	....	....	....	....	....	....	....	....	....	....	....	....	7	19.99
1912	....	....	....	4	5	1	....	....	....	....	....	....	....	....	10	23.85
1913	....	4	5	7	2	1	....	....	....	....	....	....	....	....	19	22.67
I × II F1																
1911	....	....	....	1	4	1	1	....	....	....	....	....	....	....	7	24.23
I × II F2																
1912	....	....	1	....	1	5	....	2	2	2	2	....	2	....	17	27.69
I × II F3																
1913	....	....	....	1	2	4	5	8	19	7	2	2	1	....	51	27.43

TABLE 2

Variety I, Variety II and Hybrid—Length

Designation	Class centers in mm. 37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	No. of plants	Mean flower-size
Variety I																				
1913	....	....	....	....	....	....	....	....	....	....	2	1	1	....	1	....	2	1	8	50.73
Variety II																				
1913	....	. .	3	2	7	5	1	....	1	....	....	....	....	....	....	....	....	....	19	41.34
I × II F3																				
1913	3	1	....	....	1	4	6	4	6	6	8	5	2	3	1	....	....	1	51	45.19

TABLE 3

Variety I, Variety III and Hybrids—Spread

Designation	Class centers in mm. 13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	No. of plants	Mean flower-size
Variety I																							
1910	....	....	....	....	....	....	....	....	....	....	....	....	....	....	6	....	....	....	....	....	....	6	26.93
1911	....	....	....	....	....	....	....	....	....	....	....	....	....	....	5	....	....	....	....	....	....	5	27.20
1912	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1	4	5	32.73
1913	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1	3	2	2	....	....	8	30.10
Variety III																							
1910	....	8	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	8	14.03
1911	....	5	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	5	13.85
1912	....	....	....	5	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	5	16.24
1913	1	5	7	5	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	18	15.17
I × III F1																							
1911	....	....	....	....	....	....	....	3	....	....	....	....	....	....	....	....	....	....	....	....	....	3	19.98
III × I F1																							
1911	....	....	....	....	....	....	1	2	6	3	2	....	....	....	....	....	....	....	....	....	....	14	21.83
I × III F1																							
1913	....	....	....	....	....	....	....	....	....	4	1	1	1	3	....	....	....	....	....	....	....	10	24.33

TABLE 4

Variety I, Variety III and Hybrid—Length

Designation	Class centers in mm. 28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	No. of plants	Mean flower-size
Variety I																													
1913	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	2	1	1	....	1	....	2	1	8	50.73
Variety III																													
1913	1	1	3	1	....	5	5	2	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	18	32.58
I × III F1																													
1913	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1	....	1	3	2	2	1	....	....	....	....	....	....	10	45.29

TABLE 5

Variety II, III and Hybrids—Spread

Designation	Class centers in mm. 13	14	15	16	17	18	19	20	21	22	23	24	25	No. of plants	Mean flower-size
Variety II															
1910	....	....	....	....	....	....	....	5	1	....	....	..	....	6	20.29
1911	....	....	....	....	..	..	..	7	..	..	..	....	.	7	19.99
1912	....	....	....	....	.	....	....	....	....	....	4	5	1	10	23.85
1913	....	..	....	..	..	....	..	....	4	5	7	2	1	19	22.67
Variety III															
1910	....	8	....	....	..	..	....	....	....	....	....	..	..	8	14.03
1911	....	5	..	....	..	....	..	....	....	....	..	..	....	5	13.85
1912	....	....	....	5	....	..	..	..	....	....	....	..	....	5	16.24
1913	1	5	7	5	..	..	....	..	..	..	.	..	.	18	15.17
II × III F1															
1911	....	....	2	4	2	2	1	....	....	....	....	.	....	11	16.48
III × II F1															
1911	....	....	5	7	3	4	3	....	....	....	..	..	....	22	17.67
II × III F2															
1912	..	....	..	....	..	1	4	5	1	....	....	.	.	11	19.58
III × II F2															
1912	....	1	1	2	2	4	1	....	..	..	..	..	....	11	16.90
III × II F2															
1913	..	....	....	....	1	3	4	3	3	3	2	....	1	20	20.61

TABLE 6

Variety II, Variety III and Hybrid—Length

Designation	Class centers in mm. 26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	No. of plants	Mean flower-size
Variety II																						
1913	....	....	....	....	....	....	....	..	....	....	....	....	....	3	2	7	5	1	..	1	19	41.34
Variety III																						
1913	....	....	1	1	3	1	..	5	5	2	....	....	....	....	....	....	....	..	....	....	18	32.58
III × II F2																						
1913	1	....	1	....	....	1	1	5	2	2	2	2	1	2	....	....	....	....	....	....	20	34.43

Since the number of plants involved in the above tables is so small, it is, from one point of view, rather useless thus to express the final results of this experiment which has extended over four years and in connection with which such a considerable number of measurements have been obtained. The demand for large F_2 and later hybrid generations, to give an approximation of the desired complex ratios, was early met in experiments concerning the inheritance of what may still be called "qualitative" characters and recently the investigation of "quantitative" characters has been attempted upon a similar scale. With reference to certain of the "qualitative" characters investigated I have no basis for judging the extent and influence of the environmental factors operative, nor as to the modification of the reported results that would have been produced had careful measurements been made. Data which have been submitted, however, leave no room for doubt in my own mind that investigations on the inheritance of flower-size demand the recognition of certain definite criteria and that the results of such investigations are vitally influenced by inherent as well as externally induced physiological states peculiar to the plant. Thus it remains to be seen if as many as 800 plants are necessary to establish the validity of an expanded Mendelian notation in F_2 of a flower-size hybrid, whether the 40,000 to 80,000 measurements, seemingly essential to a fair expression of results, can be accumulated. In other words, the experiment with which this paper deals has been a partially successful effort to measure many flowers on a few plants with the thought that the conception of flower-size would thus be approximately perfect for a few, rather than certainly imperfect for many plants. It is undeniably true that the number of plants is smaller than it should be, and it is perfectly evident that if the flowers on a larger number of plants cannot be correctly measured the attempt is not worth making. Further, it has been ascertained that the general method of measurement can be greatly improved upon. The attempt to make measurements of flower-size in the field is next to useless where more than fifty plants in a single group are grown. In a flower-size investigation now in progress all the flowers which the plant produces, from the first flower to the flowers on the plant two months later, are picked off and preserved in liquid to be measured in the laboratory. A random selection and careful measurement of fifty flowers among all these flowers picked off from each plant should, then, give a fair approximation of the situation with reference to the flower-sizes involved. It is hoped that the parents and three hybrid generations can be grown together during the coming season.

Two points were emphasized in the previous communications dealing with the experiment on the inheritance of flower-size in *N. acuminata* hybrids. First, it was demonstrated that, with reference to the range of variation of the flowers borne on a single plant of one of its parents, the F_1 range was distinctly greater than the parental range. Further, of all the flowers produced by all the various hybrid plants, the largest flowers were as large as the largest of all the flowers produced on plants of the large-flowered parent. This was also true for the smallest flowers. Nothing more was claimed as to the variability of flower-size in the F_1 *N. acuminata* hybrids. It was not stated that the range of the F_1 populations was greater than the range of the populations of the corresponding parents. In respect to this particular point, however, the above frequency distributions are at least interesting. In variety I and variety III (table 3), the large-flowered and small-flowered varieties and the F_1 hybrids between them in 1911, 1912, and 1913, the F_1 range is throughout greater than the range of either parent in corresponding years. The F_1 between varieties II and III in 1911, when compared with the parents of the same year (table 5), serves to emphasize this point, as does the F_1 of the cross between varieties I and II (table 1). The number of plants is obviously inadequate, yet there is here an indication again that the question of the extent of variation in F_1 is still an open one. It might be of some significance if, in place of examining only one-eighth as many F_1 plants as F_2 plants, an example of the usual procedure, the number of individuals in these two generations were made more nearly equal in favor of the larger number (cf. Shull, 1914, p. 131).

The second point which was taken up in the earlier reports had to do with the inadequate nature of the expanded Mendelian notation as an explanation of the inheritance of quantitative characters and other complex situations. An apparently widespread doubt as to its adequacy in such connections makes it possible to note that nothing during the two years since the publication of the original reports has made untenable our position on this subject therein stated (cf. Castle, 1915, p. 97). Shull (1914) feels that we have demonstrated a notable increase in variability as to flower-size in the F_2 generation previously reported on (Goodspeed, 1913) and reports that we "refused to ascribe this greater variability to Mendelian segregation." The actual statement made was that the greater variability of F_2 as compared with F_1 "appears to make it plain that segregation does occur" (*ibid.*,

p. 173) but that this situation might be dependent upon various conditions external to the experiment and that the Mendelian explanation, while obviously applicable, was of somewhat doubtful practical value. It was suggested that the actual significance of this apparent increase in variability in F_2 might lie in an increased fluctuating variability due to environmental causes. A somewhat detailed study of the parents under strikingly different conditions of culture showed that ordinary greenhouse treatment resulted in a small but distinct increase in the length of corolla tube as compared with that of sister plants in the field. The most important factor considered in connection with the effort to show that the increased variability in F_2 was due to non-inherited effects was the influence of the age of the plant, etc., in determining the size of the flowers borne on a plant. A discussion of these points is given in a paper to follow (Goodspeed and Clausen, 1915). In the earliest report, dealing with flower-size in F_1 of these hybrids, emphasis was laid upon the fact that all the fully opened flowers were taken on each day of measurement from the various plants and measured. Thus, for the F_1 previously reported upon and contained in the foregoing tables, the "age of plant" and the "age of flower" factor (Goodspeed and Clausen), which otherwise might have accounted for the degree of variation in F_1, were both eliminated. The importance of attempting to do away with the decrease in flower-size brought about by the presence of maturing seed capsules on the plant was imperfectly appreciated up to this past season. Thus, without attempting to indicate directly the situation, as has been done for other species of *Nicotiana* in another paper (*loc. cit.*), it may simply be said that a certain proportion of the increase in variability of the F_2 population of the cross I $\times$ II 1912 (table 2) and the III $\times$ II 1912 (table 5) was due to non-inherited effects. The distributions of the parent and F_1 populations in 1912 and 1913 are much more nearly representative and accurate, since it was possible to measure approximately all the flowers produced.

Leaving out of account such situations as the striking difference between reciprocal F_2 hybrids (table 5, II $\times$ III, 1912, and III $\times$ II, 1912) as due to the small number of plants involved, the foregoing tables seem to indicate the following facts:

The F_1 generation of the *N. acuminata* flower-size hybrids shows a range of variation as great as and often greater than the parent plants in corresponding years. The mean flower-size may be approximately the mean of the parent flower-sizes or it may not.

The F_2 populations in some cases show a remarkable increase in the range of variability as compared with the parents and F_1 hybrids. This range in no case is equal to the total combined range over which the variability of both parents extend. Thus in some cases the means of individual F_2 plants may be as small as or smaller than the mean of the smallest-flowered parent plant, but in no case, for the same F_2 distribution, is the mean flower-size of the largest-flowered F_2 plant as great as the mean of the largest-flowered parent individual. Indeed, in only one case do F_2 individuals approach the size of the largest-flowered parent plants and at the same time show individuals with means within the small-flowered parental range. As has above been noted, a certain proportion of this increase in variability in F_2 populations may be ascribed to the active interference of various external and internal factors attending development. It is significant, in this connection, that the flower-size of the F_2 hybrids shows in the greater number of cases an increase of variability toward the smaller end of the parental range.

Though a number of F_3 families were grown, it was not found possible to measure flowers on more than one group of plants. The mean flower-size, for spread of flowers, of the F_2 plant from which this F_3 population was derived was 24.89 mm., while the mean corolla spread of the 51 F_3 plants was 27.43 mm. The range of this F_3 population for spread of flowers was not as great as the range of the 17 F_2 plants of the previous year, though it is approximately the same so far as the limits of the range are concerned. The mean—27.43 mm. —is approximately the same as the 1913 F_2 mean of 27.59 mm. It is entirely possible that of many other F_3 families some might have shown significant differentiation but the relatively few measurements made upon three other F_3 families showed in general a range corresponding to that of the one F_3, the distribution of which is given in table 1. The range of this F_3 with reference to length of corolla tube shows an extraordinary extent, yet the mean is approximately the same as the mean between the parents.

Transmitted February 11, 1915.

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NOTES ON THE GERMINATION OF TOBACCO SEED, II

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In a previous report (Goodspeed, 1913) an outline was given of the possibly important supplementary results to be obtained by comparing the rates of germination and the amount of germination of the seed used in breeding experiments to produce the pedigreed plants. The results of the germination tests therein tabulated indicated that there were, in the first place, distinctions between the rates of germination of the seed of reciprocal hybrids and, in the second, that the rates and possibly also the amounts of germination of the seed of F_1 and F_2 hybrids were significantly differentiated. The present paper aims to present further evidence in support of these previous suggestions and conclusions. The relation between the age of the seed of certain of our pure-line cultures, and of the seed of hybrids made between them, and the viability of this seed, was also taken up in the previous communication. It will be further considered in what follows.

The tables below give the amount and time of germination of the seed of various pure lines and hybrids of *Nicotiana* under their numbers in the University of California Botanical Garden (U. C. B. G.). A list of the species employed, together with the hybrids between them, follows in the order in which they appear in the various tabulations.

30/06—"White Tobacco" (cf. Setchell, 1912, p. 7).
17/07—*Nicotiana rustica* var. *texana* (*ibid.*, p. 15).
16/07—*N. rustica* var. *scabra* (*ibid.*, p. 14).
68/07—*N. angustifolia* (*ibid.*, p. 9).
22/07—*N. Tabacum* var. *macrophylla* (*ibid.*, p. 8).
78/05—*N. Tabacum* "Maryland" (*ibid.*, p. 5).
72/05—"Cavala" (*ibid.*, p. 5).
H 23—30/06 × 22/07.

H 24—22/07 × 30/06.
143/06—*N. multivalvis* (*ibid.*, p. 27).
H 46—16/07 × 17/07.
H 47—17/07 × 16/07.
35/05—*N. quadrivalvis* (*ibid.*, p. 27).
60/07—*N. Bigelovii* (*ibid.*, p. 25).
H 5—22/07 × 68/07.
H 15—68/07 × 22/07.
H 7—72/05 × 78/05.
H 8—78/05 × 72/05.

The method of conducting the germination tests and the conditions under which they took place were identical with those previously described (Goodspeed, 1913, p. 202). The great majority of the tests were made under my direction by Miss Minnie Yonge during February and March, 1914. The criterion of germination was a trifle different from that of previously reported experiments in that the first appearance of the caulicle without the cracked seed coats, rather than the appearance of both caulicle and cotyledons, was taken to constitute germination. Over 10,000 seeds were used.

The tables that follow (tables I and II) supplement to a certain extent the evidence concerning the relation of the age of tobacco seed and its viability as given in the earlier report (*ibid.*, p. 215). Sixteen species, varieties, and hybrids are added to the list of those tested previously (*ibid.*, p. 210) and repetitions of earlier tests are shown in two cases.

TABLE I

Year	Plant designation	Number of seeds that germinated on the days indicated															Per cent germinated, average duplicate tests
		4	5	6	7	8	9	10	11, 12	13	14	15	16	17	18	19	20	
1906	30/06	....	....	...	....	....	....	1	....	....	....	....	6	3	4	....	3	17.5
		....	....	....	....	....	....	....	....	1	1	2	2	2	6	....	4	
1907	30/06	....	....	....	....	4	8	14	27	8	6	1	1	2	5	....	3	83.0
		....	....	....	....	....	5	6	26	9	6	6	2	2	2	....	2	
1908	30/06	....	....	....	....	5	11	14	24	5	8	....	2	1	2	....	....	80.0
		....	....	....	....	4	16	31	16	4	3	2	5	2	4	....	1	
1909	30/06	....	....	....	....	....	....	1	6	4	16	17	11	11	15	....	3	87.0
		....	....	....	....	....	....	2	14	8	16	19	9	9	12	....	1	
1909	17/07	....	24	12	20	13	3	1	....	1	....	...	....	....	....	....	....	71.5
		....	22	12	15	12	5	1	....	2	....	....	....	....	....	....	....	
1909	16/07	....	7	6	32	27	15	2	....	5	....	....	....	....	....	....	....	96.0
		....	11	12	40	18	12	3	....	....	....	....	....	....	....	....	....	
1909	68/07	....	....	....	16	76	1	....	....	....	....	....	....	....	....	....	....	86.0
		....	....	....	32	44	3	....	....	....	....	....	....	....	....	....	....	
1909	22/07	....	....	....	35	47	1	....	....	....	....	....	....	....	....	....	....	87.0
		....	....	....	37	52	2	....	....	....	....	....	....	....	....	....	....	
1909	78/05	....	....	....	....	....	....	90	5	....	....	....	....	....	....	....	....	95.0
		....	....	. ..	....	....	....	90	1	4	....	....	....	....	....	....	....	
1909	72/05	....	2	....	....	59	....	32	....	....	....	....	....	....	....	....	....	93.5
		....	....	....	....	66	....	27	....	1	....	....	....	....	....	....	....	
1910	17/07	....	46	36	9	1	1	....	....	....	....	....	....	....	....	....	....	96.0
		....	50	33	10	3	....	....	....	2	....	....	....	....	1	....	....	
1910	16/07	....	23	21	27	9	6	3	....	....	3	....	....	....	1	....	....	94.0
		....	26	20	25	10	7	3	....	3	....	....	....	....	....	.. .	....	
1911	16/07	....	3	10	18	11	23	13	....	6	5	...	2	....	2	....	....	93.5
		....	3	10	12	15	28	10	....	11	....	....	....	. ..	1	....	....	

TABLE I—(*Continued*)

Year	Plant designation	Number of seeds that germinated on the days indicated																Per cent germinated, average tests duplicate
		4	5	6	7	8	9	10	11, 12	13	14	15	16	17	18	19	20	
1911	F_2 H 23 P_{31} P_{25}	....	....	....	....	....	....	2	12	14	18	14	18	3	....	8	....	88.5
		....	....	....	....	....	....	4	13	9	14	14	15	9	....	9	1	
1911	F_2 H 23 P_{31} P_{22}	....	....	....	....	....	....	1	53	23	9	4	3	1	....	....	....	95.5
		....	....	....	....	....	....	1	30	24	23	12	4	1	....	1	1	
1911	F_2 H 24 P_{34} P_{20}	....	....	1	3	21	....	40	15	5	5	2	1	....	....	....	....	88.0
		....	....	2	1	10	....	45	15	4	3	1	....	....	....	....	2	
[1]1911	F_2 H 23 P_{13} P_{24}	....	....	....	....	....	....	12	11	2	....	....	....	....	....	....	....	78.1
1911	F_2 H 24 P_{34} P_{23}	....	....	....	....	3	....	30	13	3	14	6	4	5	2	....	....	76.5
		....	....	....	....	4	....	32	6	9	6	5	....	11	1	....	....	
1911	F_2 H 24 P_6 P_2	....	....	....	....	....	....	1	2	1	3	3	....	4	3	7	2	25.0
		....	....	....	....	....	....	....	....	....	2	1	2	3	2	5	9	
1911	F_2 H 24 P_{34} P_{20}	....	....	....	....	....	....	9	2	4	3	4	8	4	1	2	1	33.0
		....	....	....	....	....	....	8	....	2	2	3	2	5	1	2	3	
1911	F_2 H 24 P_6 P_3	....	....	....	....	....	1	1	22	30	18	13	6	....	....	7	....	98.5
		....	....	....	....	....	....	....	18	16	27	6	18	....	....	12	....	
1911	[2]143/07 P_5	96	4	....	....	....	....	....	....	....	....	....	....	....	....	....	....	98.5
		90	7	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
1911	H 46	....	5	22	15	22	....	20	6	....	4	....	2	....	....	....	....	98.0
		....	4	13	25	21	....	24	7	....	6	....	....	....	....	....	....	
1911	H 47	29	44	13	....	3	....	....	....	....	....	....	....	....	....	....	....	91.0
		26	60	5	....	1	....	....	1	....	....	....	....	....	....	....	....	
1912	F_3 H 24 P_{34} P_{23} P_2	....	....	....	18	32	27	8	7	4	1	....	1	....	....	1	....	98.5
		....	....	....	17	34	25	11	6	3	1	....	....	....	....	1	....	

[1] Only 32 seeds used in test.

TABLE I—(*Concluded*)

Year	Plant designation	Number of seeds that germinated on the days indicated															Per cent germinated, average duplicate tests
		4	5	6	7	8	9	10	11, 12	13	14	15	16	17	18	19	20	
1912	[2]35/05 a (5) P_8	62	38	....	....	....	....	....	....	....	....	....	....	....	....	....	....	100.0
		72	28	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
1912	[2]60/07 a P_4 P_6	21	52	13	....	5	....	....	6	....	....	....	....	....	....	....	....	97.0
		24	53	10	....	5	....	....	5	....	....	....	....	....	....	....	....	
1912	17/07	....	89	2	....	....	1	....	....	....	....	....	....	....	....	....	....	95.0
		....	98	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
1912	16/07	95	....	....	1	....	1	....	....	....	....	....	....	....	....	....	....	97.0
1912	F_3 H 23 P_{13} P_{24} P_1	....	....	....	3	23	35	19	10	4	....	....	....	....	....	1	....	93.5
		....	....	....	3	10	28	25	22	2	2	....	....	....	....	1	....	
1912	F_1 H 46 P_{20}	20	40	12	4	6	....	3	3	....	1	....	1	....	....	....	....	81.5
		21	40	10	7	5	....	3	2	....	1	....	3	1	....	....	....	
1912	F_1 H 46 P_7	2	....	80	7	....	2	....	1	....	....	....	....	....	....	....	....	91.5
		1	....	73	11	....	3	....	2	....	....	1	....	....	....	....	....	
1912	F_1 H 47 P_{20}	2	....	74	16	....	3	....	....	....	....	....	....	....	....	....	....	94.0
		1	....	64	20	....	6	....	2	....	....	....	....	....	....	....	....	
[3]1912	F_1 H 47 P_2	2	....	50	41	....	8	....	2	....	....	....	....	....	....	....	....	86.0

[2] Marked germination the third day.

[3] 120 seeds used.

TABLE II

Year	Plant designation	Number of seeds that germinated on the days indicated															Per cent germinated, average duplicate tests
		4	5	6	7	8	9	10, 11	12	13	14	15	16	17	18	19	20	
1909	17/07	....	24	12	20	13	3	1	....	1	....	....	.. .	....	.. .	....	....	71.5
		....	22	12	15	12	5	1	....	2	..	. .	....	....	....	....	....	
1909	16/07	....	7	6	32	27	15	2	....	5	....	....	....	....	....	....	....	96.0
		....	11	12	40	18	12	3	....	...	. .	....	....	.	.	....	....	
1909	72/05	....	2	....	....	59	....	....	32	....	....	..	.. .	....	.. .	....	....	93.5
		....	....	....	....	66	....	....	27	....	1	....	....	....	....	....	....	
1909	78/05	....	....	....	....	....	....	90	5	....	...	....	....	....	....	....	....	95.0
		....	....	....	....	....	....	90	1	4	....	. .	....	....	....	....	....	
1909	68/07	....	....	....	16	76	1	....	....	....	....	....	....	....	....	....	....	86.0
		....	....	....	32	44	3	....	....	....	....	....	....	....	....	....	....	
1909	22/07	....	....	....	35	47	1	....	....	....	....	....	....	....	....	....	....	87.0
		....	....	....	37	52	2	....	....	....	....	....	....	....	....	....	....	
1909	30/06	....	....	....	....	.. .	....	1	6	4	16	17	11	11	....	15	3	87.0
		....	....	....	....	....	....	2	14	8	16	19	9	9	....	12	1	

TABLE II*a*

	Year	Plant designation	Av. per cent germinated	
(1)	1906	30/06	17.5	
	1907	30/06	83.0	
	1908	30/06	80.0	
	1909	72/05, 30/06, 17/07, 16/07, 68/07, 22/07, 78/05	87.9	
	1910	17/07, 16/07	95.0	
	1911	16/07, 143/07	96.0	
	1912	17/07, 16/07	96.0	
(2)	1906	30/06	17.5	
	1907	30/06	83.0	
	1908	30/06	83.0	
	1909	30/06	87.0	
(3)	1909	16/07 and 17/07	96.0	71.5
	1910	16/07 and 17/07	94.0	96.0
	1911	16/07	93.5	.....
	1912	16/07 and 17/07	97.0	95.0

TABLE II*b*

Plant designation	Year	Amount of germination, per cent	Duration of significant germination	Maximum germination
30/06	1906	17.5	8 days (+)	16th day of test
110/05	1906	59.0	9 days	7th day of test
78/05	1906	25.5	25 days	29th day of test
30/06	1907	83.0	10 days	12th day of test
110/05	1907	70.5	9 days	7th day of test
78/05	1907	77.5	15 days	11th day of test
30/06	1908	80.0	11 days	11th day of test
110/05	1908	74.5	11 days	7th day of test
22/07	1908	88.0	9 days	7th day of test
30/06	1909	87.0	9 days	15th day of test
110/05	1909	79.5	13 days	7th day of test
78/05 (1913)	1909	71.5	9 days	11th day of test
78/05 (1914)	1909	95.0	3 days	10th day of test
22/07 (1913)	1909	96.0	11 days	9th day of test
22/07 (1914)	1909	87.0	3 days	8th day of test
68/07	1909	86.0	3 days	8th day of test
72/05	1909	93.5	5 days	8th day of test
17/07	1909	71.5	6 days	5th day of test
16/07	1909	96.0	7 days	7th day of test
110/05	1910	26.5	5 days	11th day of test
78/05	1910	62.5	15 days	11th day of test
22/07	1910	89.5	7 days	7th day of test
17/07	1910	96.0	5 days	5th day of test
16/07	1910	94.0	9 days	5th day of test
110/05	1911	84.0	5 days	7th day of test

Table 11*b*—(*Concluded*)

Plant designation	Year	Amount of germination, per cent	Duration of significant germination	Maximum germination
78/05	1911	95.0	7 days	11th day of test
22/07	1911	98.0	5 days	7th day of test
16/07	1911	93.5	10 days	9th day of test
143/07	1911	98.5	2 days	3rd day of test
110/05	1912	88.5	11 days	7th day of test
78/05	1912	98.5	9 days	9th day of test
22/07	1912	96.0	9 days	5th day of test
17/07	1912	95.0	2 days	5th day of test
16/07	1912	97.0	2 days	4th day of test
35/05 (*a*) (5)	1912	100.0	2 days	3rd day of test
60/07 (*a*)	1912	97.0	6 days (+)	5th day of test

Table I details the results of germination tests, some of which are expressed more briefly in tables II, II*a*, and II*b*. The data submitted furnish a confirmation of the results previously reported, which demonstrated that tobacco seed five years old and older will give a relatively high percentage of germination under controlled conditions. As to whether deterioration in viability is a gradual process or has a sharp end-point after which viability is greatly reduced we can, again, make no generally applicable answer. The combined evidence furnished by the previous experiments and those above submitted seems to make it clear that for certain species and varieties the end-point is abrupt, while for others deterioration as to viability is a gradual process. This distinction can be made even within a species, since the various *N. Tabacum* varieties differ from one another as to the amount of seed which becomes functionless in each succeeding year. In this connection attention should be called both to the uniformity and to the lack of uniformity in the rates of germination of the seed of various varieties within a given species. Take, first, the six varieties of *N. Tabacum,* the seed of which is dealt with in this and in the preceding report. Table II*b* combines results given in Table 3 of the preceding report (Goodspeed, 1913, p. 210) with certain of those included in Table I and lays emphasis upon the number of days during which a significant amount of germination took place as well as the day, during the extent of the germination test, upon which the greatest amount of germination was noted. As Table II*b* shows, there is an extraordinary uniformity as to the day upon which the greatest amount of germination took place. There is, in a number of cases, a character-

istic day and it is peculiar to both older and younger seed. Thus for *Nicotiana angustifolia* (110/05) the seed aged two, three, five, six, seven, and eight years gave the greatest amount of germination on the seventh, while the four-year-old seed gave the greatest amount on the eleventh day of the germination tests. Likewise, in the case of *N. Tabacum* "Maryland", the eleventh day showed the greatest amount of germination for the seed of four different years, while the youngest seed germinated most heavily on the ninth day. In the same way the seed of *N. Tabacum* var. *macrophylla* showed, on the average, maximum germination on the seventh day. Table II shows that for five-year-old seed of five *N. Tabacum* varieties three gave maximum germination on the eighth day, one on the eleventh, and one on the fifteenth, while of two varieties of *N. rustica* one showed the greatest amount of germination on the fifth and the other on the eighth day. With reference, further, to these *N. rustica* varieties (16/07 and 17/07) as shown in Table II*b*, seed of three different ages germinated most heavily on the fifth day, while seed of four different years of 16/07 germinated, on the average, later during the germination tests. Both the total amount of germination and the length of time during which significant germination took place varies more greatly for the different species and varieties used. There seems to be, however, some significant differentiation in this latter respect among the varieties of *N. Tabacum* and *N. rustica*. Thus seed of 17/07 completes its significant germination in a shorter space of time than does 16/07. Similarly 78/05 and 110/05 exhibit, on the average, a greater duration of germination than does the seed of the other *N. Tabacum* varieties.

As will be seen by a reference to the earlier communication (1913, p. 215), certain apparently significant facts were revealed when the germination of parental vs. hybrid seed, through a number of generations, was tested and the results compared. The first part of Table V expresses in condensed form the data given in the earlier paper (*l.c.*, p. 216). H 18 represents the cross 110/05 × 78/05; H 20 its reciprocal. The striking difference between the results of the germination test carried out with 1909 78/05 in 1913 and the one made with the same seed in 1914 will be explained by reference to the earlier paper (*l.c.*, p. 221). As may be seen, H 18 and H 20 are not significantly differentiated in any striking fashion with reference to the duration of germination and as to the day during the extent of the test upon which the maximum amount of germination took place. The average total amount of germination of the parents, including

TABLE III

Year	Plant designation	Number of seeds that germinated on the days indicated																Per cent germinated, average duplicate tests
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1909	68/07	....	....	....	....	16	76	1	....	....	....	....	....	....	....	....	....	86.0
		....	....	....	....	32	44	3	....	....	....	....	....	....	....	....	....	
1909	22/07	....	....	....	....	35	47	1	....	....	....	....	....	....	....	....	....	87.0
		....	....	....	....	37	52	2	....	....	....	....	....	....	....	....	....	
1909	H 5	....	....	....	....	7	81	....	2	....	....	....	....	....	.	....	....	92.0
		....	....	....	....	15	76	....	3	....	....	....	....	....	....	....	....	
1909	H 15	....	....	....	....	11	55	....	4	....	....	....	....	....	....	.	....	70.0
1909	78/05	....	....	....	....	....	....	90	5	....	....	....	....	....	....	....	....	95.0
		....	....	....	....	....	....	90	1	4	....	....	....	....	....	....	....	
1909	72/05	2	....	....	59	....	....	32	....	....	....	....	....	....	....	....	....	93.5
		....	....	....	66	....	....	27	....	1	....	....	....	....	....	....	....	
1909	F_1 H 7 P_1	....	....	....	....	41	....	....	35	....	2	....	....	....	....	....	....	77.0
		....	....	1	....	44	....	....	31	....	....	....	....	....	....	....	....	
1909	F_1 H 8 P_1	....	....	3	....	84	....	....	9	....	....	....	....	....	....	....	....	97.5
		....	....	4	....	86	....	....	9	....	....	....	....	....	....	....	....	
1910	17/07	....	....	46	36	9	1	1	....	....	....	....	....	....	....	....	....	96.0
		....	....	50	33	10	3	....	....	....	....	....	....	....	....	....	....	
1910	16/07	....	....	23	21	27	9	6	3	....	....	3	....	....	....	1	....	94.0
		....	....	26	20	25	11	7	3	....	3	....	....	....	....	....	....	
1911	H 46	....	....	5	22	15	22	.	20	6	....	4	....	2	....	....	....	98.0
		....	....	4	13	25	21	....	24	7	....	6	....	....	....	....	....	
1911	H 47	29	....	44	13	....	3	....	....	....	....	....	....	....	....	....	....	91.0
		26	....	60	5	....	1	....	....	1	....	....	....	....	....	....	....	
1912	F_1 H 46 P_{20}	....	20	40	12	4	6	....	3	3	....	1	....	1	....	....	....	81.5
		....	21	40	10	7	5	....	3	2	....	1	....	3	1	....	....	
1912	F_1 H 46 P_7	....	2	....	80	7	....	2	....	1	....	....	....	....	....	....	....	91.5
		....	1	....	73	11	.	3	....	2	....	....	1	....	....	....	....	
1912	F_1 H 47 P_{20}	....	2	....	74	16	....	3	....	....	....	....	....	....	....	....	....	94.0
		....	1	....	64	20	....	6	....	2	....	....	....	....	....	....	....	
1912	F_1 H 47 P_2*	....	2	....	50	41	....	8	....	2	.	....	....	....	....	....	....	86.0

* Only 120 seeds used.

1906 to 1912 seed in the case of 78/05 and 1905 to 1912 seed in the case of 110/05, is approximately 70 per cent, whereas the seed of H 18 and H 20, five years old, germinated 89.5 per cent and 96.5 per cent respectively. The five-year-old seed of 78/05 germinated 71.5 per cent in the 1913 test and 95 per cent in the 1914 test, while the five-year-old seed of 110/05 germinated only 79.5 per cent. Comparisons between the germination of the seed of other *N. Tabacum* varieties and that of the F_1 seed of hybrids made between them are, some of them, more significant (tables III, IV, and V). It is certainly remarkable that the six-year-old seed of 68/07, 22/07, H 5 (22/07 × 68/07), and H 15 (68/07 × 22/07) should show almost exactly the same duration of germination and that, for all four, the day upon which maximum germination took place should be the same. Too much emphasis cannot be laid upon the duration of germination in this case, since the 1913 test of the 1909 22/07 seed showed this to be eleven days as contrasted with three days for the 1914 test of this same seed. Attention must, however, be called to the fact that the important portion of the 1913 germination of the 1909 22/07 seed took place within five days (cf. Goodspeed, *l.c.*, p. 211), while the remainder of the seed that germinated after these five days accounts approximately for the difference between the total amounts of germination of the five-year-old seed and the six-year-old seed of 22/07. Contrasted with the similarities in the duration of germination and the day upon which germination took place in the greatest amount, we have a distinct difference between the reciprocal hybrids, H 5 and H 15, as to the total amounts of germination—i.e., 92 per cent for H 5 and only 70 per cent for H 15. The seed produced by F_1 of the cross 78/05 × 72/05 and its reciprocal can, likewise, be distinguished by differing amounts of total germination. Thus while the six-year-old seed of the parents, 78/05 and 72/05, germinated 95 per cent and 93.5 per cent respectively, the 1909 seed of F_1 H 7 (72/05 × 78/05) germinated 77 per cent and corresponding seed of its reciprocal (F_1 H 8) germinated 97.5 per cent. It is also important to note that the germination of 1909 72/05 is characterized by two maxima of germination—the sixth and the ninth day of the test—and that F_1 H 7, also, shows maxima of germination—the seventh and the tenth day of the test. As noted above, 72/05 is the female parent of the hybrid H 7. Similarly, comparing the germination of 1909 F_1 H 8 and 1909 78/05, the correspondence between the two is striking in that they both have but one day of maximum germination, and thus that the

TABLE IV

Year	Plant designation	Number of seeds that germinated on the days indicated																Per cent germinated, average duplicate tests
		5	6	7	8	9	10, 11	12	13	14	15	16	17	18	19	20	21	
1909	30/06	....	....	....	....	....	1	6	4	16	17	11	11	....	15	3	....	87.0
		....	....	....	....	....	2	14	8	16	19	9	9	....	12	1	....	
1909	22/07	....	.. .	35	47	1	....	....	.. .	....	. .	....	....	.. .	. .	....	....	87.0
		....	....	37	52	2	....	. .	....	....	....	....	....	....	....	....	....	
1911	F_2 H 23 P_{31} P_{25}	.. .	....	....		....	2	12	1	18	14	18	3	.	8	....	....	88.5
		....	....	....	..	...	4	13	9	14	14	15	9	.. .	9	1	....	
1911	F_2 H 23 P_{31} P_{22}	....	....	....	....	....	1	53	23	9	4	3	1	.. .	....	....	....	95.5
		...	....	..	.. .	....	1	30	24	23	12	4	1	....	1	1	....	
1911	F_2 H 24 P_{34} P_{20}	. .	1	3	21	40	15	5	5	2	1	....	....	....	....	....	....	88.0
		....	2	1	10	45	15	4	3	1	....	....	. .	.. .	. .	2	....	
1911	F_2 H 24 P_{34} P_{23}	....	...	....	3	....	30	13	3	14	6	4	....	5	2	....	....	76.5
		....	....	. ..	4	....	32	6	9	6	5	....	.. .	11	1	....	....	
1911	F_2 H 24 P_6 P_2	....	....	....	....	....	1	2	1	3	3	....	....	4	3	7	2	25.0
		....	....	....	.	...	....	...	....	2	1	2	. .	3	2	5	9	
1911	F_2 H 24 P_{34} P_{20}	....	....	....	....	....	9	2	4	3	4	8		4	1	2	1	33.0
		....	....	....	....	....	8	. .	2	2	3	2	....	5	1	2	3	
1911	F_2 H 24 P_6 P_3	....	....	....	....	....	1	1	....	22	30	18	13	6	.	....	7	98.0
		....	....	....	....	....	....	....	....	18	16	27	6	18	....	....	12	
1912	F_3 H 24 P_{34} P_{23} P_2	....	....	18	32	27	8	....	7	4	1	....	1	....	....	1	....	98.5
		....	....	17	34	25	11	....	6	3	1	.	.. .	....	....	1	....	
1912	F_3 H 23 P_{13} P_{24} P_1	....	....	3	23	35	19	....	10	4	....	....	. .	....	....	1	....	93.5
		....	....	3	10	28	25	....	22	2	2	.. .	....	....	....	1	....	

TABLE V

Plant designation	Year	Amount of germination, per cent	Duration of significant germination	Maximum germination on
110/05	1905 to 1912	69.3 (av.)	9 days (av.)	8th day of test
78/05	1906 to 1912	71.7 (av.)	18 days (av.)	13th day of test
110/05	1909	79.5	13 days	7th day of test
78/05	1909 (1913)	71.5	25 days	15th day of test
78/05	1909 (1914)	95.0	3 days	11th day of test
H 20	1909	96.5	7 days	9th day of test
H 18	1909	89.5	9 days	7th day of test
110/05	1910	26.5	7 days	11th day of test
78/05	1910	62.5	29 days	11th day of test
F_1 H 20 P_{26}	1910	98.5	7 days	9th day of test
F_1 H 18 P_{49}	1910	97.5	3 days	5th day of test
110/05	1911	84.0	7 days	7th day of test
78/05	1911	95.0	7 days	11th day of test
F_2 H 20 P_{26} P_{11}	1911	91.5	16 days	11th day of test
F_2 H 20 P_{26} P_{25}	1911	95.0	11 days	7th day of test
F_2 H 18 P_{49} P_{25}	1911	99.0	5 days	7th day of test
*F_2 H 18 P_{49} P_{24}	1911	99.0	5 days	7th day of test
17/07	1910	96.0	4 days	5th day of test
16/07	1910	94.0	10 days	7th day of test
H 46	1911	98.0	10 days	8th day of test
H 47	1911	91.0	6 days	5th day of test
F_1 H 46 P_{20}	1912	81.5	13 days	5th day of test
F_1 H 46 P_7	1912	91.5	9 days	6th day of test
F_1 H 47 P_{20}	1912	94.0	8 days	6th day of test
F_1 H 47 P_2	1912	86.0	9 days	6th day of test
68/07	1909	86.0	3 days	8th day of test
22/07	1909	87.0	3 days	8th day of test
H 5	1909	92.0	4 days	8th day of test
H 15	1909	70.0	4 days	8th day of test
30/06	1909	87.0	11 days	15th day of test
22/07	1909	87.0	3 days	8th day of test
F_2 H 23 P_{31} P_{25}	1911	88.5	10 days	16th day of test
F_2 H 23 P_{31} P_{22}	1911	95.5	8 days	12th day of test
F_2 H 24 P_{34} P_{20}	1911	88.0	9 days	9th day of test
F_2 H 24 P_{34} P_{23}	1911	76.5	11 days	11th day of test
F_2 H 24 P_6 P_2	1911	25.0	12 days	20th day of test
F_2 H 24 P_{34} P_{20}	1911	33.0	12 days	11th day of test
F_2 H 24 P_6 P_3	1911	98.0	12 days	15th day of test
F_3 H 24 P_{34} P_{23} P_2	1912	98.5	9 days	8th day of test
F_3 H 24 P_{13} P_{24} P_1	1912	93.5	9 days	9th day of test
78/05	1909	95.0	3 days	11th day of test
72/05	1909	93.5	4 days	6th day of test
F_1 H 7 P_1	1909	77.0	4 days	7th day of test
F_1 H 8 P_1	1909	97.5	6 days	7th day of test

* Up to and including this record the table is based upon results given in a previous article (cf. Goodspeed, *l.c.*, p. 216), except "78/05 1909 (1914)," which is taken from table I (p. 235).

extent of really significant germination is approximately the same. 78/05 represents the parent of the hybrid H 8. A corresponding situation holds for the seed of the F_1 *N. rustica* hybrids. Thus H 47 and F_1 H 47 P_{20} and P_2 correspond in their manner of germination to 17/07, which was the female parent in the cross. Similarly, H 46 and F_1 H 46 P_{20} show the extended period of germination which is characteristic of 16/07, the female parent of the cross. F_1 H 46 P_7, on the other hand, compares more closely in its germination with 17/07, H 47 and F_1 H 47 P_{20} and P_2. F_1 H 46 P_7 and F_1 H 47 P_{20} show a relatively high percentage of total germination, while F_1 H 46 P_{20} and F_1 H 47 P_2 show a relatively low percentage.

Tables IV and V exhibit the germination of 30/06 and 22/07 and the seed of F_2 and F_3 of hybrids made between them. H 23 represents the cross 30/06 $\times$ 22/07, and H 24 its reciprocal. The seed resulting from the cross-pollinations and the seed from F_1 plants were not available. As seen in Table I and Table IV, the seed of 30/06 has a characteristically extended period throughout which germination continues to take place. This fact may have led to the statement made by Setchell (1912, p. 8) concerning the "poor germinating power" of this *N. Tabacum* variety. If under the most favorable conditions significant amounts of germination are taking place only after nearly three weeks in the germinating case, the viability of such seed sown in soil would have appeared to be low, especially as most of the other *N. Tabacum* varieties complete their germination in less than two weeks.

The F_3 and F_4 seed of H 23 and H 24 are rather distinctly differentiated among themselves in a number of ways. It is characteristic of the parent 22/07 to have a limited duration of significant germination (cf. Goodspeed, *l.c.*, p. 210), which is in contrast to the extended period of germination characteristic of the other parent, 30/06 (Table I). Thus the day upon which maximum germination takes place occurs in the case of 22/07 relatively early in the test, while in the case of 30/06 it occurs relatively late. The seed of F_2 H 23 P_{31} P_{25}, F_2 H 23 P_{31} P_{22}, and F_2 H 24 P_6 P_3 in a general way parallels 30/06 in these respects, while the seed of F_2 H 23 P_{31} P_{21}, F_3 H 24 P_{34} P_{23} P_2, and F_3 H 24 P_{13} P_{24} P_1 corresponds more nearly to 22/07. Similarly the seed of F_2 H 24 P_{34} P_{23} occupies roughly an intermediate position between 22/07 and 30/06, while within the germinating period of three weeks the seed of F_2 H 24 P_6 P_2 and F_2 H 24 P_{34} P_{20} germinated only 25 per cent and 33 per cent respectively and the period during which

germination probably would have continued would have considerably exceeded even the extended period characteristic of 30/06.

The germination of hybrid seed was again undertaken this past year partly in the expectation that the F_2, F_3, and F_4 seed might give some evidence of such segregation as was observed to take place among the plants grown from this seed. Thus it seemed possible that of the seed produced by a given F_2 plant a distinct portion might germinate early in the germination test and other portions have maxima of germination on later dates. It was then planned to grow separately plants from the seed that germinated in considerable amounts at distinct intervals in the test. Such groups of plants would be expected to be similar within themselves and distinct from other groups. It is evident that seed of the various pure lines of *Nicotiana* described in this and the previous report either complete their germination within three or four days with little or no scattering germination before or after this period of maximum germination, or that a few seeds germinate early in the test and their number increases on succeeding days until the maximum is reached, after which there is a corresponding gradual decrease in the number that germinate until germination is at an end. In only one case, 72/05, are there two unconnected maxima of germination, and in this case the corresponding situation evidenced by the seed of F_1 H 46 was assigned to the influence of this 72/05 parent. The germination of the F_3 and F_4 seeds of H 46 and H 47 (tables IV and V) realize, in some degree, the expected differentiation among the seeds produced by hybrid plants of F_1 and later generations. F_2 H 24 P_{34} P_{23} and F_3 H 23 P_{13} P_{24} P_1, for example, show breaks in the continuity of germination which are hardly as distinct in the germination of any of the pure lines of *Nicotiana* tested with the exception of the peculiar germination of *N. acuminata* varieties elsewhere noted (1913, pp. 207 and 210). The evidence did not, however, seem sufficiently conclusive to make it desirable to grow plants from the seedlings produced at different periods during the germination tests.

SUMMARY

The sum total of the evidence at hand concerning the germination of hybrid vs. parental seed leaves no doubt that different plants of F_1, F_2, and F_3 produce seed the germination of which is significantly differentiated as to the total amount of the seed that will germinate, or as to the length of time during which germination takes place, or as to the period, during the extent of the test, within which the maximum amount of germination occurs. Further, there is evidence that among the seeds of a single F_2 or F_3 plant a portion have a characteristic period during which they germinate, which is distinct from the period characteristic of another group of the same seed. The suggestion is made in this connection that if the plants grown from the seeds which germinated most heavily at one period were kept distinct from those resulting from seed with a different maximum of germination the plants within each group would correspond one to another in their inheritance of certain characters. Certainly the results indicate that care must be taken in pricking out seedlings from the seed pans not to discriminate in favor of the larger seedlings if the resulting plants are to exhibit a representative progeny of the parent individual (cf. Goodspeed, 1912, p. 111). Unfortunately our facilities are such that the germination, under controlled conditions, of large quantities of seed is not possible, nor is sufficient space available for growing the number of plants necessary to give final evidence on this subject. Finally, the F_1 seed from reciprocal crosses has been shown to differ with reference to its germination. In this connection, either the amount of total germinaion, or the extent of the germinating period, or the days of maximum germination peculiar to one parent have been shown also to be characteristic of the cross-pollinated seed which it bore.

Transmitted April 8, 1915.

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PARTHENOGENESIS, PARTHENOCARPY AND PHENOSPERMY IN *NICOTIANA*

BY

THOMAS HARPER GOODSPEED

Following the publication in 1909 of an article by Mrs. R. H Thomas on "Parthenogenesis in *Nicotiana*," the validity of her results—the production of parthenogenetic seed without difficulty in species and hybrids of *Nicotiana*—has been called in question by a number of investigators who have either made an exhaustive investigation of the subject as a special problem or have included a consideration of it as a preface to their general breeding experiments (cf. Wellington, 1913, Howard, 1913, Hayes and Beinhart, 1914). So far as I know, however, no one has attempted to investigate the behavior of the strains of tobacco which Mrs. Thomas actually employed in the experiments which she described. The present communication details experiments performed on a variety of *Nicotiana Tabacum*, the seed of which was obtained from Mrs. Thomas. From over 800 castrations and mutilations of buds on the plants raised from this imported seed, a little normally matured and viable seed was obtained. In over 100 cases, involving almost 200 flowers, such treated buds yielded full capsules of seed which was practically normal in appearance but which consisted, in almost all cases, of empty seed-coats only. This latter situation introduces the *possibility* that after one or two years additional cultivation in our soil and climate, the undoubted parthenocarpy of the first year, which was accompanied by the production of much "abortive" seed, may be increased to, and accompanied by, a more general parthenogenesis than was found this past year.

A complete and careful reading of Mrs. Thomas' original paper will go far toward convincing anyone that the occurrence of parthenogenesis as reported for her cultures of *Nicotiana* is not wholly the result of errors in technique and manipulation. Wellington (*loc. cit.*, p. 288) makes the obvious suggestion that Mrs. Thomas failed to notice adventitious buds which so frequently develop along with the castrated buds under the bag in *Nicotiana*. Thus he feels that the seed she found was produced not by the castrated flowers but by untreated flowers from adventitious buds which had been originally overlooked. Mrs. Thomas' report of her experiments certainly leaves much to be desired so far as essential, descriptive detail is concerned. One is, however, convinced that the ordinary precautions to prevent contamination were observed and that nothing imperfect or unusual in the method of sterilizing the instruments or trimming or bagging the inflorescences can be claimed. Further, Mrs. Thomas definitely states in her type description of technique that she opened a bag ten days after the castration and pinched off the "one or two tiny buds which were sprouting" alongside of the castrated flowers which had in that time "set seed" (Thomas, *loc. cit.*, p. 2). She thus appreciated the fact that in the ready production of adventitious buds there was a source of error for her experiments. The fact that approximately an equivalent length of time is necessary as well for the development of an open flower from a bud as for the maturing and shedding of seed from an open flower, makes it evident that at least a week and probably three times that length of time would have intervened between the ripening of the seed from castrated flowers and from overlooked, untreated flowers. It is almost inconceivable that Mrs. Thomas could have overlooked one or a number of flowers which would probably have been fully open at the time she would have expected the ripened capsules or seed from the castrated flowers to be ready for removal from the plant. Wellington (*loc. cit.*) also calls attention to the fact that "both self-fertilized and parthenogenetic blossoms produce offspring true to the mother species; and consequently an error, if it did occur, could not be detected." This statement should have little significance for Mrs. Thomas' experiments in that she found seed parthenogenetically produced on five distinct hybrid groups in F_1 and F_2. In such material parthenogenetic and self-fertilized seed should, according to current views on heredity, give different offspring.

However, she makes the following rather ambiguous remark in describing these same experiments in another publication (1913). "La Parthénogénèse ou Apogamie peut se produire dans plusieurs espèces ou variétés de *Nicotiana.* Les graines parthénogénétiques semées reproduisent identiquement l'espèce ou la variété, ou bien, si ces graines sont obtenues comme résultat d'un croisement en F_1 ou F_2, la segrégation attendue dans la couleur des fleurs se produit." Bateson, also, has confirmed Mrs. Thomas' results for the *N. Tabacum* variety which she employed in her experiments (cf. Bateson, 1913).

Mrs. Thomas, at my request, was kind enough to send me seed of the "*Nic. tabaccum* Cuba" which is referred to in her paper (p. 2). I take the liberty of quoting extracts from a letter of Mrs. Thomas' which describes the origin of the seed and adds certain details of interest. "Since then [the appearance of her original paper in the *Mendel Journal*] I have every season produced parthenogenetic seed from one or more *Nicotiana,* though the conditions for growing the plants here [Moyles Court, Ringwood] are not so favorable as at Creech (?) Grange, Wareham, where I first made trials for parthenogenesis and succeeded in so many varieties." The seed which I obtained from Mrs. Thomas was one capsule of "the original seed gathered in the Garden of Casa Loring at Malaga in 1908." In 1913 this original seed "produced two or three hundred plants" in Mrs. Thomas' garden. Her letter further states that she uses "unperforated wax-paper bags for the protection and very young buds for castration" and that parthenogenesis had been found for "the poppy and a variety of *Delphinium* as well as *Oenothera biennis* and parthenocarpy in many plants which fail to ripen their seed." Further details as to the origin of "*Nic. tabaccum* Cuba" are given in Mrs. Thomas' paper (1909, p. 2; cf. Bateson, 1913).

The record of the germination tests with this seed is unfortunately missing, but no difficulty was experienced in obtaining 95 plants in the field this past season (1914) from a small sowing of seed.

Mrs. Thomas thus describes "*Nic. tabaccum* Cuba:"

> The plant is taller than most other *N. tabaccums,* and is 6½ to 7 ft. in height, and the stems are very thick; it flowers at first in a terminal cluster and afterwards axially. The limb and tube are pure white; the corolla is sometimes four-petalled with four stamens, sometimes five-petalled with five stamens, and both forms are found on the same plant. It is a freely pollinating plant, for under protection from insects it will seed every blossom.

The average height of the 95 plants grown in our cultures this past year was approximately 2 m., the variation in height having extremes at approximately 1.5 and 3 m. The average number of leaves was 33 and the range of variation from 31 to 37, while the number of laterals to the last significant leaf was 17 with a range from 11 to 37. The stand was remarkably uniform and compared favorably in this regard with other cultures of *N. Tabacum* varieties which have been grown in the pure line for ten years. The flowers are borne in great abundance, at first from the terminal panicle and later from the many flowering laterals. In our cultures, the flowers were not "pure white" but a greenish white for the tube and a dull white with a suggestion of greenish for the limb. The color is "white" when compared with another white-flowered *N. Tabacum* variety grown in our cultures for some years—i.e., "White Tobacco," U. C. B. G. 30/06 (cf. Setchell, 1912, p. 7)—but cannot be called white in the sense that *N. sylvestris* Speg. and Comes is a white-flowered tobacco. The length of the corolla tube will average approximately 45 mm. and the diameter of the limb 25 mm. The corolla tube is slender and the infundibulum slightly swollen. The limb is broadly and shallowly lobed with the lobes unpointed. The leaves are large; the average length of the fourth leaf up being 49 cm. and its greatest breadth 24 cm. Its shape is elliptical lanceolate, broadly and decurrently auriculate at the base, with the auricles equal and broadly acuminate at the tip. A photograph of one of the plants not used in the experiments until late in the season is appended (plate 35). As noted by Mrs. Thomas, there is a striking variation in the number of flower parts. I found a number of three-parted flowers and at the opening of the flowering season almost equal numbers of four and five-parted flowers. Toward the end of the flowering season five-parted flowers predominate. Throughout this report the name given by Mrs. Thomas in her original paper—i.e., "*Nic. tabaccum* Cuba"—is employed. In the University of California Botanical Garden the plant number is 200/14.

Three points were borne prominently in mind in planning the carrying out of experiments to test for the occurrence of parthenogenesis[1] in these plants. In the first place, it seemed essential that

[1] Lacking the necessary definite cytological evidence, the term parthenogenesis is throughout employed in a more or less unrestricted sense and as equivalent to the production of normally matured, viable seed without pollination (cf. Winkler, 1908, and Coulter, 1914, p. 119.)

as much data as possible be accumulated concerning the condition of the bud to be treated and the condition of the plant at the time of treatment. Thus the length of each bud at the time of castration or mutilation was determined and recorded and the date when the first flowers opened was noted for every plant. These data give a basis for comparison as to the "age" of the plants at the time of treatment. The maximum length of unopened buds was 49 mm.—the measurement being taken from the point of union of the pedicel and calyx to the tips of the folded corolla lobes. As the tightly folded corolla lobes begin to open back, the anthers open, and I have rarely found a bud unopened and less than 48 mm. long in which pollen was being shed. The length of the buds treated seemed to be the only reliable method of distinguishing between younger and older buds. In these experiments of the first year it was desired only to determine whether the age of the bud when treated was significant for the production of parthenogenesis or parthenocarpy. It seemed also desirable that the position on the plant of the bud to be treated should be determined. Thus the records were so kept that it is possible to state whether the castration or mutilation was made upon the terminal inflorescence, on one of the four or five "bald suckers," or on some one of the lower laterals. These data are in a sense supplementary to those that describe the age of the plant, since the lower leafy laterals were in flower later than the terminal inflorescence or the bald suckers. The number of the flower parts was also determined in each case, since it seemed at least possible that the four-parted flowers might give results somewhat different from the more normally five-parted flowers.

The second point which was considered to be essential with reference to these experiments had to do with providing all possible checks upon the treatment given the buds. All doubt as to whether or not the various operations had been correctly carried out can be eliminated by saving in every bag all the flower parts. This is not easy to do when the bag must be opened to pick off adventitious buds, but it is, to my mind, an absolute necessity for any sure interpretation of results in such work. In the case of the castrations and mutilations tabulated below the corollas of the flowers originally treated were in every case available for examination when the seed was cleaned (Goodspeed, 1912, p. 129) and again when it was sown. Every corolla was examined under the dissecting microscope to determine

whether or not the castration had been complete. This examination showed that, of over 800 castrations, in only one case had anthers been overlooked. Two anthers were present on the withered corollas from the castration of two buds on plant 25 and, as will be seen, a fair proportion of good seed resulted (cf. table 1). The corollas are, in most cases, still available for examination in the seed packets together with the seed not sown.

The third essential point had to do with the technique and manipulation concerned in treating the buds. For any one familiar with the technique of making hybrids through cross-pollination the essential precautions to prevent contamination by foreign pollen become second nature. The important point, in addition to this most essential one, in such operations is the delicacy with which the manipulations can be performed to prevent injury to the flower parts not to be treated. The profusion of flowering laterals on the plants used in the experiments herein described made it necessary to cut away masses of flowers, from other inflorescences, near the bud before it could be operated upon. In a certain number of cases this was not carefully enough done and the close proximity of flowers about the bud after its castration made it seem best to discard it. A number of such doubtful castrations were, however, bagged and in one case undoubtedly self-pollinated seed in fair proportion was produced—i.e., plant 14 (table 1). The buds were, with the exception of the smallest, slightly split open along the upper third of the corolla tube and the castrations or mutilations performed through this slight opening, which was not sufficient in extent to expose the stigma. If, in picking out the anthers, the stigma was inadvertently touched by the forceps the bud was discarded.

The treatment given the 800 buds employed was of three types only. The first consisted of simple emasculation of the flower by picking off the anthers of the bud near the tops of the filaments. The second involved the removal of the stigma as well as the anthers. The stigma in such cases was pinched off with the forceps, before picking out the anthers, at the very top of the style. In the third type of treatment the stigma was pinched off, in the above manner, but the anthers were allowed to remain untouched. In such cases the tip of the style, somewhat crushed, had dried down exceedingly before any pollen could have been shed in the open flower. The last two types of treatment will hereafter be referred to as "mutilations."

No success attended a small number of attempts to cut with a razor directly across the whole flower, severing corolla, filaments and style (cf. Thomas, *loc. cit*). Such treatment caused the buds to fall within three days. The number of simple castrations far exceeded either of the other two types of treatment.

The plants of "*Nic. tabaccum* Cuba" began to flower during the first week of September, 1914, and were still in flower, though very feebly, early in February, 1915. The castrations and mutilations were begun in the case of plants 1 to 75 on the day that the first flower of the terminal inflorescence opened, or as near that date as possible. As the plants came more fully into flower as many buds as possible were treated. For the first month, flowers on each succeeding bald sucker were operated upon almost as soon as the first flowers were open. Later in the season time did not permit any such close following of the plants. The nineteen plants, 76 to 95, were growing some fifty feet away from the rest and most of them were left untouched until they were "beginning to go off their fullest bloom" (Thomas, *loc. cit.*, p. 5). These nineteen plants were set out into the field somewhat later than the rest and came into flower somewhat later also. When their flowers were first operated upon, the terminal inflorescence and many of the upper laterals bore many partially matured seed-capsules. No seed was shed from untreated, unbagged flowers on any of the plants until the last week in October.

The bags which evidently contained seed were taken from the plant at once. In practically all cases the seed had been shed from the capsules which had opened normally. The cleaning of the seed was done with the utmost care and I cannot feel that any contamination could have occurred in this connection. As a result of the cleaning, it was evident that a very considerable number of castrated and mutilated flowers had given seed, a part of which was apparently normally matured and the majority of which consisted of nothing but empty seed-coats. It was out of the question to attempt germination tests of all the seed thus available. According to our usual method of making such tests with tobacco seed (Goodspeed, 1913), the number of seeds to be handled would in this case have approximated 10,000 and probably the germination of double that number would have been found to be necessary. It was thus judged best to adopt the following method for determining which of the packets of seed contained only empty seed-coats and which contained any apparently

normally matured seed. From 200 to 300 seeds were placed in a watch glass and covered with strong Eau de Javelle. In twenty-four hours the characteristic dark brown color of the seed-coats was bleached out and when placed on a dark background the question as to whether or not the seed-coats were empty could be answered at a glance. In every case a control was made by similarly treating the selfed seed of "*Nic. tabaccum* Cuba" obtained this past season. This control served to show that the length of time for which the bleaching solution was allowed to act was not sufficient to dissolve the endosperm and embryo and served also as an aid in distinguishing between the normal and abortive seed produced by the treated flowers. Although the chalky white seed containing endosperm could be distinguished at once from the transparent functionless seed, quantities of the bleached seed were examined under magnification in every case. The slightest trace of endosperm could thus be detected and by gentle pressure on the cover-glass over the preparation the embryos of the normal seeds could be forced out of the endosperm through the softened seed-coats. In a number of cases seed, which according to the bleaching test consisted of empty shells only, was germinated as a check upon the method but no germination was noted at the end of three weeks. All seed packets which were indicated by the bleaching test to contain seed with endosperm or endosperm and embryos were also germinated. In every case all the seed remaining in the packets was sown according to the method elsewhere described (Goodspeed, 1913).

The following table indicates so far as possible all significant facts concerning the 112 treatments of flowers which yielded abortive or normal seed, together with the proportions of seed with endosperm and embryos and seed with endosperm only.

TABLE 1

Plant number	Date of first flower	Date of treatment	Treatment: Castrated	Treatment: Castrated and pinched off stigma	Treatment: Pinched off stigma only	Number of buds	Length of buds in mm.	Number of stamens: Stamens[1] 4	Number of stamens: Stamens 5	Position on plant: Terminal inflorescence	Position on plant: Bald sucker	Position on plant: Lower laterals	Per cent of seeds with: Endosperm and embryo	Per cent of seeds with: Endosperm only
1	9/20	9/24	×	....	....	3	28*	1	2	×	.....	.....	0	0
		10/2	×	....	....	1	32	....	1	....	4th	.....	1	0
		11/2	×	....	....	1	27	1	....	....	.....	9th	0	0
2	9/24	10/2	×	....	....	1	34	....	1	....	2nd	.....	0	0
3	10/1	10/21	×	....	....	1	45	1	....	×	.....	.....	0	0
		10/21	....	×	....	1	30	....	1	....	1st	.....	0	0
		10/21	....	×	....	1	36	....	1	....	.....	5th	3	0
4	9/18	10/21	×	....	....	2	33†	....	2	....	.....	5th	0	0
5	9/17	9/18	×	....	....	3	28‡[2]	....	3	×	.....	.....	0	0
6	9/15	9/15	×	....	....	5	42‡	5	....	×	.....	.....	0	0
		9/25	×	....	....	1	35	....	1	....	3rd	.....	0	0
		10/30	×	....	....	1	21	....	1	....	4th	.....	0	0
7	9/12	9/12	×	....	....	4	31‡	3	1	×	.....	.....	0	0
8	9/8	9/25	×	....	....	2	38†	2	....	....	.....	4th	0	0
		9/25	×	....	....	2	48†	1	1	....	3rd	.....	0	0
9	9/8	9/18	×	....	....	3	43‡	2	1	....	2nd	.....	0	0
11	9/12	9/12	×	....	....	1	42	....	1	×	.....	.....	0	0
		9/26	×	....	....	4	31‡	4	....	....	1st	.....	0	0
		9/26	×	....	....	2	28†	....	2	....	2nd	.....	0	0
		9/26	×	....	....	1	26	1	....	....	.....	4th	0	0
		10/30	?	....	....	1	46	....	1	....	.....	6th	0	0
14	9/8	9/8	×	....	....	4	25‡	3	1	×	.....	.....	0	0
		9/26	?	....	....	2	46†	2	....	....	1st	.....	34	0
17	9/13	9/15	....	....	×	1	48	....	1	×	.....	.....	0	0
18	9/22	9/26	×	....	....	3	41‡	1	2	×	.....	.....	0	0
20	9/17	9/18	×	....	....	2	33†	2	....	×	.....	.....	0	0
		9/26	×	....	....	1	39	....	1	....	1st	.....	0	0
21	9/25	9/26	×	....	....	3	30‡	2	1	×	.....	.....	0	0
		9/26	×	....	....	1	41	1	....	....	2nd	.....	0	0
		9/26(x)	×	....	....	1	38	....	1	....	.....	4th	0	0
23	9/17	9/26	×	....	....	2	40‡	1	1	....	1st	.....	0	0
24	9/24	9/26	×	....	....	4	27‡	3	1	×	.....	.....	0	0
		9/26	×	....	....	1	37	....	1	....	1st	.....	0	0
		9/29	×	....	....	1	32	1	....	....	.....	4th	0	0
		9/29	×	....	....	1	28	1	....	....	.....	5th	0	0
25	9/11	9/26	?	....	....	2	48†	2	....	....	1st	.....	51	0

* all buds. † both buds. ‡ buds averaged.

[1] Flowers with 4 stamens were, with very few exceptions, four-parted throughout and similarly for the flowers with 5 stamens.

[2] By the use of the word "averaged" it is, in all cases, meant that all the buds treated were within 2 mm. either larger or smaller than the size stated.

Table 1—(*Continued*)

Plant number	Date of first flower	Date of treatment	Treatment: Castrated	Treatment: Castrated and pinched off stigma	Treatment: Pinched off stigma only	Number of buds	Length of buds in mm.	Number of stamens: Stamens[1] 4	Number of stamens: Stamens 5	Position on plant: Terminal inflorescence	Position on plant: Bald sucker	Position on plant: Lower laterals	Per cent of seeds with: Endosperm and embryo	Per cent of seeds with: Endosperm only
28	9/8	9/29	×	....	....	2	46†	1	1	....	.....	4th	0	0
29	9/26	9/29	×	....	....	1	44	....	1	....	1st	.....	0	0
32	9/7	9/29	×	....	....	2	40†	....	1	....	.....	5th	0	0
33	9/17	9/18	×	....	....	4	30‡[2]	....	4	×	.....	.....	0	0
34	9/10	9/29	×	....	....	1	43	....	1	....	1st	.....	2	0
36	9/16	9/18	×	....	....	1	48	....	1	×	.....	.....	7	0
38	9/25	9/29	×	....	....	2	42†	....	2	×	.....	.....	0	0
		9/29	×	....	....	1	42	1	....	....	1st	.....	0	0
		9/29	×	....	....	1	43	1	....	....	2nd	.....	0	0
		9/29[3]	....	....	×	1	—	—	—	....	3rd	.....	0	0
40	9/20	9/24	×	....	....	4	37‡	3	1	×	.....	.....	0	0
		9/29	×	....	....	1	45	1	....	....	2nd	.....	0	0
41	9/13	9/15	×	....	....	2	41‡	2	....	×	.....	.....	0	0
		9/29	×	....	....	1	43	....	1	....	.....	3rd	0	0
		9/29	×	....	....	3	41‡	2	1	....	.....	4th	0	0
43	9/16	9/18	×	....	....	3	38‡	3	....	×	.....	.....	0	0
		9/29	×	....	....	3	30‡	2	1	....	.....	3rd	0	0
44	9/18	9/29	×	....	....	1	43	....	1	....	1st	.....	0	0
		9/29(x)	×	....	....	2	32†	2	....	....	2nd	.....	0	0
45	9/20	9/21	×	....	....	3	39‡	1	2	×	.....	.....	0	0
		10/1	×	....	....	1	47	1	....	....	1st	.....	0	0
		10/1	×	....	....	1	35	1	....	....	2nd	.....	0	0
49	9/21	9/24	×	....	....	3	24‡	2	1	×	.....	.....	0	0
50	10/30	10/30	×	....	....	2	31†	1	1	×	.....	.....	0	0
53	10/1	10/1	×	....	....	3	36‡	3	....	×	.....	.....	0	0
56 Axis No. 1	9/28	10/1	×	....	....	3	41‡	2	1	×	.....	.....	0	0
(56) Axis No. 2	9/30	10/1	×	....	....	3	24‡	2	1	×	.....	.....	0	0
58	9/30	10/1	×	....	....	3	30‡	3	....	×	.....	.....	0	0
		10/1	×	....	....	1	27	1	....	....	1st	.....	0	0
		10/1	×	....	....	1	27	1	....	....	3rd	.....	0	0
		10/1	×	....	....	2	46‡	1	....	....	.....	4th	0	0
59	9/29	10/1	×	....	....	2	43‡	2	....	×	.....	.....	0	0
60	9/15	10/1	×	....	....	1	49	1	....	....	3rd	.....	0	0
61	9/19	10/1	×	....	....	2	28†	1	1	....	1st	.....	0	0

† both buds. ‡ buds averaged.

[1] Flowers with 4 stamens were, with very few exceptions, four-parted throughout and similarly for the flowers with 5 stamens.

[2] By the use of the word "averaged" it is, in all cases, meant that all the buds treated were within 2 mm. either larger or smaller than the size stated.

[3] In this case, the only instance of such treatment, a large bud was allowed to open and shed pollen normally and two hours after the opening of the anthers the stigma alone was pinched off.

TABLE 1—(*Concluded*)

Plant number	Date of first flower	Date of treatment	Treatment: Castrated	Treatment: Castrated and pinched off stigma	Treatment: Pinched off stigma only	Number of buds	Length of buds in mm.	Number of stamens: Stamens 4	Number of stamens: Stamens[1] 5	Position on plant: Terminal inflorescence	Position on plant: Bald sucker	Position on plant: Lower laterals	Per cent of seeds with: Endosperm and embryo	Per cent of seeds with: Endosperm only
62	9/21	10/1	×	....	....	2	29†	1	1	×	.....	.....	0	0
63	9/28	10/30	×	....	....	1	27	....	1	×	.....	.....	0	0
		10/30	×	....	....	1	41	....	1	....	2nd	.....	0	20
		10/30	....	×	....	1	42	1	....	....	3rd	.....	0	0
66	9/18	9/21	×	....	....	2	23†	2	....	×	.....	.....	0	0
67	9/13	9/15	×	....	....	4	24‡[2]	3	1	×	.....	.....	0	6
		10/1	×	....	....	3	46‡	....	3	....	.....	4th	0	0
76	9/27	10/13	×	....	....	1	38	1	....	×	.....	.....	0	0
		10/13	×	....	....	1	38	....	1	....	3rd	.....	0	0
78	9/26	10/13	×	....	....	1	36	1	....	....	2nd	.....	0	0
		10/13	....	....	×	1	30	....	1	....	3rd	.....	0	0
		10/13	....	....	×	1	34	1	....	....	.....	8th	3	0
		10/13	×	....	....	1	47	1	....	....	.....	9th	0	0
		10/13	×	....	....	1	47	1	....	....	.....	10th	0	0
80	9/25	10/13	×	....	....	1	31	....	1	....	1st	.....	0	0
81	10/10	11/5	×	....	....	2	42†	1	1	....	5th	.....	0	0
82	9/25	10/13	×	....	....	1	38	....	1	×	.....	.....	0	0
		10/13	×	....	....	1	38	....	1	....	5th	.....	0	0
83	9/18	10/21	×	....	....	1	32	....	1	....	1st	.....	0	0
84	9/15	10/21	×	....	....	1	32	1	....	....	5th	.....	2	2
85	9/26	10/23	×	....	....	3	34‡	3	....	....	1st	.....	0	0
		10/23	×	....	....	1	38	....	1	....	3rd	.....	0	0
		10/23	×	....	....	2	36†	....	2	....	5th	.....	0	0
		10/23	×	....	....	1	29	....	1	....	.....	6th	0	0
		10/23	×	....	....	1	33	....	1	....	.....	8th	0	0
86	10/8	10/24	×	....	....	1	42	....	1	....	1st	.....	0	0
		10/24	....	×	....	1	31	....	1	....	.....	6th	3	0
87	10/4	10/24	×	....	....	1	47	....	1	....	1st	.....	0	0
		10/24	×	....	....	1	33	....	1	....	5th	.....	0	0
89	9/26	10/29	×	....	....	1	24	....	1	....	2nd	.....	0	0
Axis No. 1														
		10/29	×	....	....	1	26	....	1	....	4th	.....	2	0
		10/29	×	....	....	1	26	....	1	....	5th	.....	0	0
		10/29	×	....	....	1	35	....	1	....	.....	6th	0	0
(89)		10/29	×	....	....	1	41	....	1	....	1st	.....	0	0
Axis No. 2														
		10/29	×	....	....	1	41	1	....	....	2nd	.....	2	0
		10/29	×	....	....	1	40	....	1	....	3rd	.....	0	0
		10/29	×	....	....	1	35	....	1	....	4th	.....	0	0
		10/29	×	....	....	1	29	....	1	....	.....	5th	0	0
92	9/28	10/29	×	....	....	1	31	....	1	....	3rd	.....	0	0
95	9/29	11/5	×	....	....	1	46	....	1	....	1st	.....	0	0
		11/5	×	....	....	2	46†	....	2	....	4th	.....	0	0

† both buds. ‡ buds averaged.

[1] Flowers with 4 stamens were, with very few exceptions, four-parted throughout and similarly for the flowers with 5 stamens.

[2] By the use of the word "averaged" it is, in all cases, meant that all the buds treated were within 2 mm. either larger or smaller than the size stated.

Table 2 which follows gives the length and breadth of the seed of a few of the plants mentioned in table 1. The averages are based on approximately 20 measurements in each case. For two of the castrations which produced some normally matured seed (plants 14 and 36, below) measurements are given for the length and breadth of the normal seeds and also of the abortive seed. Many of the seeds with "empty seed-coats" were of full size, the figures given representing average sizes. It was possible to select from the abortive seed of one capsule over 75 seeds which were indistinguishable in size from the seed resulting from self-fertilization.

TABLE 2

Plant No.	Date	Treatment	Average length, mm.	Average breadth, mm.	Remarks
16	.....	self-fertilized	0.39	0.25	Normal seeds that germinated over 80 per cent.
14	9/26	castrated(?)	0.37	0.24	Castration judged to be contaminated (cf. p. 254)—seeds with endosperm and embryos.
	9/26	castrated(?)	0.26	0.20	Seeds of same doubtful castration —empty seed-coats only.
21	9/26(x)	castrated	0.26	0.19	Empty seed-coats only.
36	9/18	castrated	0.38	0.25	Seeds with endosperm and embryos.
	9/18	castrated	0.27	0.21	Empty seed-coats only.
44	9/29(x)	castrated	0.27	0.19	Empty seed-coats only.

It is thus possible to state that of some 800 castrations and mutilations divided approximately evenly between the 95 plants of "*Nic. tabaccum* Cuba" grown this past year, there were 112 instances, involving nearly 200 flowers, in which one or more fruits developed to normal size and matured seed, almost all of which was normal in appearance, though small in size, and a small proportion of which was normal in every way including the presence of endosperm and embryos.

During the past ten years a very considerable collection of *Nicotiana* species and varieties has been grown in the pure line in the Botanical Garden of the University of California. They have been fully described elsewhere (Setchell, 1912). Every year two or three plants of each of the species and varieties listed below have been

tested by castration and mutilation experiments for the presence of parthenogenesis. On the appearance of Mrs. Thomas' original paper, Professor Setchell made an additional series of experiments to confirm his previous observations. This past season, in connection with the experiments detailed above on "*Nic. tabaccum* Cuba," I made over four hundred castrations of flowers of *N. Tabacum* var. *macrophylla*, *N. angustifolia*, *N. Tabacum* "Maryland" and *N. sylvestris*. The number of plants in each case was twenty-five and the castrations were made just as the plants were "going out of their first bloom," which, according to Mrs. Thomas, is the most favorable period for the production of parthenogenetic seed. The plants were also so thoroughly cut back that the castrated flowers were the only ones left on the plant; all maturing and mature seed capsules were also removed. This treatment, as noted by Howard (1913 p. 41) and in our cultures also, usually induces heavy and rapid seed production. In addition, the occurrence of parthenogenesis has continually been tested for in connection with the making of a very considerable number of hybrids between many of the species and varieties listed below. Whenever a castration, preliminary to cross-pollination, is made, another bud or two on a different part of the plant is also castrated and left unpollinated at the time of making the cross. We may further add to this summary, as being significant, the hybrids between *N. sylvestris* and various *N. Tabacum* varieties. The F_1 hybrids are completely self-sterile since, in our cultures of them, no normal pollen is produced. Over 500 baggings have been made of F_1 flowers and a great variety of efforts have been made to stimulate seed production according to the methods described by Wellington (1913). In no case did parthenocarpy or parthenogenesis result. It is important to note in this connection that Mrs. Thomas reports an F_1 hybrid between "*N. sylvestris* and *N. tabaccum* Cuba" and an F_2 of the cross "*N. sylvestris* by *N. affinis*" as producing parthenogenetic seed. I have never been able to make the latter hybrid successfully and have yet to see a strictly fertile F_1 species hybrid involving *N. sylvestris* as a parent. In our cultures the many hybrids that have been made have never given any evidence of the production of parthenogenetic or apogamous seed in the sense that hybrids have bred true to the maternal parent from F_1 on through later generations.

Partial List of Species and Varieties Used in Testing for Parthenogenesis

A description of them is to be found in Professor Setchell's paper (1912).

Nicotiana Tabacum "Brazilian"	Nicotiana rustica (seven varieties)
Nicotiana Tabacum "Cavala"	Nicotiana Langsdorfii
Nicotiana Tabacum "Maryland"	Nicotiana paniculata
Nicotiana Tabacum var. calycina	Nicotiana alata
(Nicotiana Tabacum) "White Tobacco"	Nicotiana acuminata (three varieties)
Nicotiana Tabacum var. macrophylla	Nicotiana Bigelovii
Nicotiana angustifolia	Nicotiana sylvestris
Nicotiana Tabacum var. macrophylla purpurea	Nicotiana tomentosa
"Nic. tabaccum Cuba"*	Nicotiana glauca

* Described in this paper but not in Setchell (1912).

The total number of flowers involved in these various efforts to produce parthenogenetic seed is well over 1500 and the number of plants concerned over 450. In not a single instance was any seed of any sort produced, and only very rarely did the capsules remain attached for over two weeks and when, in a few cases, they did persist the result was a shrunken, misshapen capsule containing nothing but the dried-up ovules in their entirely immature form. The falling of castrated flowers in such experiments on *Nicotiana* species is very striking. Howard (1913) well describes the difference in this respect between the result of self- or cross-pollination and the result of castration:

> A great difference was found between the capsules formed from castrated flowers and those formed by ordinary pollination. In the latter case the capsule remains firmly attached to the plant. No difficulty is experienced in removing or replacing bags, and the peduncle would have to be broken before the capsule could be removed. This is always the case whether the flowers be self- or cross-pollinated. The capsules of the castrated flowers, on the other hand, although they also become swollen at first and simulated the fertilized ones, were very easily detached from the plant. It was exceedingly difficult to remove the bags, which finally had to be cut away carefully. The capsules thus exposed to the air were easily blown or knocked off.

Wellington (1913) has given a detailed summary of the literature dealing with the problems of parthenogenesis on the plant side, and no further review of it need be given here. I wish, however, to call attention to two additional references which are of particular interest here. First the experiments of Howard referred to above, which are detailed as a preface to the description of breeding experiments with 51 varieties of *N. Tabacum*. The experiments in castration and mutilation included considerably over 5000 flowers and some apparently parthenogenetic seed was produced. Second, the experiments of Hartley (1902) with tobacco, which are especially important in con-

nection with the efforts of Wellington to stimulate the production of parthenogenesis and parthenocarpy. These two references will be mentioned below in greater detail.

Viable seed supposedly parthenogenetically produced has been obtained by Professor East in the case of the following crosses: *N. paniculata* × *N. alata* var. *grandiflora*, *N. rustica* × *N. Tabacum*, *N. Tabacum* × *N. Bigelovii*, *N. paniculata* × *N. Langsdorfii*, *N. paniculata* × *N. longiflora*, *N. paniculata* × *N. Forgetiana*, *N. Bigelovii* × *N. sylvestris*, and *N. Tabacum* var. *lanciflora* × *N. alata* var. *grandiflora* (Wellington, 1913, and East, 1910). The supposition that these cross-pollinations induced, by the "extraordinary irritation of foreign pollen" (East, *loc. cit.*), the production of apogamic or parthenogenetic seed was suggested by the fact that certain of the seeds produced plants "like the mother species and also true hybrids" and that certain of them gave plants "like the mother species and no true hybrids" and that certain others of them gave "no true hybrids on one occasion but did produce true hybrids on other occasions" (Wellington *loc. cit.*). Species crosses made by Gärtner also gave seed in a few cases that produced the mother species and also true hybrids (Burbridge, 1877). This phenomenon has apparently occurred a sufficiently large number of times to preclude the possibility that errors in technique were the cause. Wellington in his experiments produced "abortive seed probably without embryos" by singeing young buds, by exposing young plants to chloroform gas, "cutting away a portion of the pistil and pollinating the stub both with and without the accompaniment of a germinative fluid" and by "shortening the pistils (?) of a flower and grafting the stigma end of another pistil on to the stub and pollinating the same." In only one doubtful case was seed produced "by the simple methods of emasculation and decapitation of blossoms."

As a result of the very numerous castration experiments of Howard (*loc. cit.* 1910) on a plant of *N. Tabacum*—Type 9 (cf. Howard and Howard, 1910)—one castrated flower produced a capsule "the seed of which germinated and produced plants similar to type 9." "In 1911, again, on a plant of type 9, one capsule containing seed was found in about 100 castrated flowers." Three other capsules in every way normally matured and apparently containing seed were produced, in Howard's experiments, as a result of castrating flowers of another type of *N. Tabacum*. These capsules were, however, lost before any definite determinations were made as to their contents.

Hartley (1902, p. 15) makes the following statement concerning certain of his interesting experiments on flowers of "Cuban Tobacco (*Nicotiana tabaccum*)": "Of 60 emasculated flowers that had their stigmas covered with substances other than pollen, 14 set fruits, while of 20 that were emasculated but never pollinated, 2 set fruits." Continuing, he says:

> As a general thing the capsules, resulting from flowers that were not pollinated, and likewise those resulting from flowers whose stigmas were covered with some substance other than pollen, contained only small, compressed, undeveloped seeds, but the two pods obtained . . . by treating the fully receptive stigmas with magnesium sulphate contained some spherical seeds of almost full size which looked like good seeds, but when cut into proved to be hollow spheres.

Mrs. Thomas' results have been detailed elsewhere in this paper.

Summarizing these previous efforts to obtain parthenogenetic seed in *Nicotiana*, we may state that of the many thousands of castrations and mutilations of flowers concerned in all the experiments above noted, only two capsules containing viable seeds were produced. This statement leaves out of account Mrs. Thomas' remarkable results. In only a few cases also, and then only after artificial stimulation, did these castrated flowers produce normal fruits. In the majority of cases where normal fruits were thus produced, abortive seed was formed in the mature capsules. Accompanying the general failure of castrated and mutilated flowers to mature fruits, the early falling of the flowers has attracted attention. It is therefore remarkable that in the experiments, some of which are shown in table 1, there should be a striking absence of this tendency of the castrated or mutilated flowers to fall soon after the operation. Although over seven times as many treated flowers fell as remained normally attached to the plant until maturity, still, except in the case of a complete severance of the floral organs just above the ovary, a great majority of the treated flowers remained on the plant for a period twice or three times as long as is normal for treated flowers of any other species or variety of *Nicotiana* in our cultures. Nearly 115 treated flowers, most of them following castration, matured normal fruits, ripened their seed and shed it from the dehiscing capsules. In nine of the 112 cases tabulated in table 1, some seeds of normal size and appearance were produced. The remainder of the seed produced in these 112 instances was less than normal size but the great majority of it was normal in appearance—small, plump seed. I have

found that seed of this same size and appearance, produced occasionally in our cultures of other *N. Tabacum* varieties, will give up to or over fifty per cent germination. The small seed, normal in appearance, in almost all cases was found to consist of entirely empty seed-coats. In three cases, however, considerable amounts of endosperm could be distinguished under magnification but, though the seeds were carefully dissected, no trace of embryos could be found.

I know of no special term to describe the production of seed which appears to be normal but consists of empty seed-coats only—i.e., the type of seed usually referred to when the word "abortive" is used. At Professor Setchell's suggestion the term *phenospermy* is proposed to cover this production of "abortive seed." The seed produced by Wellington and Hartley as a result of stimulation following castration may thus be spoken of as *phenospermic*.

The total number of seeds, all of which were of size similar to the self-fertilized seed of "*Nic. tabaccum* Cuba" and a portion of which, after bleaching with Eau de Javelle, were seen to contain endosperm and embryos normally formed, was approximately 50. Unfortunately, a rather large percentage of this number was destroyed in the bleaching test. As the seed was shaken from the seed packets into the watch glasses (cf. p. 256) a large part of the heavier normal seed fell out in each case. Only 18 seeds, among those remaining in the seed packets which were determined by the bleaching test to contain normal seed, were available for germination. After three weeks eight of them had germinated and six seedlings are normally developing. Over twenty germination tests of seed from packets which gave no indication of normal seed with the bleaching test have not shown any signs of germination. The self-fertilized seed of "*Nic. tabaccum* Cuba" gave germination tests averaging 83 per cent after three weeks, and practically all the seeds that did not germinate were phenospermic (cf. Thomas, 1909, p. 4).

It is worthy of note that, of the castrations and mutilations which produced normal or phenospermic seed, approximately 30 per cent occurred on the terminal inflorescence; 50 per cent on the terminal inflorescence and four or five bald suckers normal to this variety, and 20 per cent only on the lower leafy laterals. Castrations and mutilations made within two weeks after the opening of the first flower gave normal or phenospermic seed in 65 instances, as compared with 56 instances for operations performed more than two weeks after the opening of the first flower. There was also no significant

increase in successful treatments on the plants which were allowed to "go off their fullest bloom" before starting experiments on their flowers. In the total series of 800 experiments buds as short as 11 mm. and as long as 48 mm., were used for castration and mutilation. The number of buds under 35 mm. in length which gave fruits and seeds following treatment is almost identical with the number of buds over 35 mm. The size of the bud to be treated has, then, little significance for the production of fruits and seed following castration or mutilation. Similarly, four- as compared with five-parted flowers are not significant for the production of parthenogenetic or phenospermic seed, since the various castration and mutilation experiments described in table 1 involve approximately equal numbers of the two types of flowers. However, seed containing endosperm and embryos, or endosperm alone, seems to have resulted, in practically all cases, from the more normally formed, five-parted flowers. Similarly the use of a single bud on an inflorescence for treatment seems, as was perhaps to have been expected, to have been more efficient for the production of normal seed than the castration or mutilation of more than one bud on an inflorescence. Of the total 800 operations less than one third involved the pinching off of the stigma as well as emasculation. Similarly only one tenth of the total number involved the pinching off of the stigma only. Thus it is significant that of the nine cases in which normally formed seeds were produced, three should represent treatments in which the stigma was removed. In general for the production of parthenogenetic and phenospermic seed, simple castration seems most effective. It seems probable that certain of the stimulating and irritating agents used by Hartley and Wellington would be more effective for parthenogenesis in "*Nic. tabaccum* Cuba" than in the varieties which they employed. No experiments of this sort were attempted.

I have assumed throughout that parthenogenetic seed was actually produced in a few cases following the castration or mutilation of flowers borne on the variety of *N. Tabacum* which Mrs. Thomas used in her much more generally successful experiments concerning parthenogenesis in *Nicotiana.* If there were not a very remarkable tendency in this *N. Tabacum* variety to mature normal fruits and phenospermic seed following castrations and mutilations of flowers, or, on the other hand, if anything approximating full capsules of normal seed had resulted in a number of cases from such treatment of flowers, I should feel entirely willing to assign the production of

the relatively small amount of viable seed that did mature, to errors in technique. I have attempted to show that the experiments were planned and carried out with a full knowledge of the sources of error and with every effort to guard against their becoming operative. Where contamination was suspected to have occurred in the short interval between the act of castration or mutilation and the bagging of the treated bud, it, as noted above, was discarded in all but a few cases. In these few cases, bags were at once put on and in table 1 is shown the result in the three instances in which the fruits matured—i.e., plant 11, 10/30, plant 14, 9/29 and plant 25, 9/26. In one case nothing but phenospermic seeds were formed, and in the other two hundreds of normal viable seeds. This simply means that if the viable seed, which was produced following apparently unimpeachable treatments, was due to chance pollination by wind, or the shaking of neighboring flowers' pollen into the bud, larger amounts of viable seed should have been present than were actually found in the nine unsuspected cases of seed production. I cannot feel that the instruments were non-sterile in as many as nine distinct cases. But apart from these considerations, reference to Hartley's paper (*loc. cit.*) will give information based upon experiments concerning the effect of premature pollination which must correspond to the general observations of anyone who has been concerned in hybridization experiments with tobacco. Hartley found, that of buds plentifully pollinated with mature, fresh pollen from the same plants, those pollinated more than one day before opening matured 4 per cent of fruits which contained no viable seed, and those pollinated one day or less than one day before opening, matured 86 per cent of fruits which contained viable seed. It is thus rather inconceivable that chance pollination of small, castrated buds could have resulted in fertilization in such a distinct number of cases. I do not think that light would be thrown upon this particular point by delaying this report until the maturity of the seedlings produced from the parthenogenetic seeds, because the possibility that pollen of a foreign species was present and thus that plants of a hybrid nature and appearance should result, is negligible. It may, in this connection, be stated that cross-pollination of *N. Tabacum* "Maryland" and other *N. Tabacum* varieties made on "*Nic. tabaccum* Cuba" have given normally filled capsules of viable seed.

The facts taken together seem to indicate that three stages are to be observed in the extent to which "*Nic. tabaccum* Cuba" will

mature normal seeds without pollination either accompanied by the supposed stimulation of mutilation or not. First the production of phenospermic seeds. Second, the production of phenospermic seeds which contain a greater or lesser amount of cellular structure, rich in starch and proteid, and taken to represent endosperm (cf. Woodburn, 1911, etc.). Third, the production of seed normal in appearance, containing endosperm and embryos fully developed and capable of germination and initiating the growth of normal seedlings. To my mind the production of a considerable number of parthenocarpic fruits containing phenospermic seed is the most significant result of the experiments above described. I desire again to call attention to the *possibility* that after one or two years of further cultivation in our cultures, the large proportion of phenospermic seeds with or without embryos may be lessened in favor of a greater proportion of entirely normal, viable seeds resulting from the castration or mutilation of flowers of "*Nic. tabaccum* Cuba." I feel, also, that this variety of *N. Tabacum* by its rather ready parthenocarpy and phenospermy as it was grown in our cultures this past year, and entirely apart from such cases of parthenogenesis as were found, furnishes a partial confirmation of Mrs. Thomas' results in similar experiments on plants of the same variety. I fully understand that this is practically equivalent to the statement that the difference in soil and possibly general climatic conditions between England and California will account for the small amount of parthenogenetic seed obtained in the experiments above described as contrasted with the frequent and ready production of such seed by the same plants as grown by Mrs. Thomas. Nothing that has been said, however, must suggest that I desire to confirm her general experimental results; results which she feels indicate that parthenogenesis is peculiar to *Nicotiana* species in general. It must, on the contrary, be emphasized that our general results point to exactly the reverse condition and that we have no reason to suppose that parthenogenesis has occurred in any of our previous cultures. Speculation, also, as to the time at which and the way in which parthenogenesis, parthenocarpy and phenospermy arise in "*Nic. tabaccum* Cuba" must be of little significance until pertinent cytological data can be accumulated. I have, further, no suggestion to offer, at the present time, as to the possible origin of this *Nicotiana Tabacum* variety which exhibits such marked divergence from the restricted method of fruit and seed production peculiar to other varieties of this species and to all other species of tobacco so far as known.

Summary

The above report has to do primarily with a white flowered variety of *N. Tabacum* the seed of which was received under the name "*Nic. tabaccum* Cuba." This is the variety in which Mrs. R. H. Thomas found parthenogenesis to be of such frequent occurrence.

1. Over 1500 attempts to produce parthenogenetic seed on a considerable number of species and varieties of *Nicotiana* have yielded entirely negative results.

2. In a considerable number of distinct hybrids made between such *Nicotiana* species and varieties and grown in some cases through five hybrid generations, no evidence has been furnished that the possible irritating and stimulating effect of cross-pollination has resulted in the production of any parthenogenetic or apogamous seed.

3. Approximately 800 castrations and mutilations of buds borne on plants of "*Nic. tabaccum* Cuba" produced over 100 normally matured fruits. Following the majority of these 800 castrations and mutilations, the flowers and maturing capsules, though ultimately they may have fallen, remained attached to the plant for much longer periods of time than is the case in other species and varieties of *Nicotiana* when similar treatment is applied.

4. In the majority of these parthenocarpic fruits empty seeds were produced in great numbers. Some were as large as the self-fertilized seed of the same plant, though the majority were smaller. Few flattened or shrivelled seeds were formed.

5. For this type of seed production, either with or without pollination, the term *phenospermy* is suggested. It is taken to be synonymous with the terms "abortive" and "empty" that have been elsewhere applied in describing such seeds.

6. Approximately 50 seeds were found in nine of the parthenocarpic fruits, some of which exhibited normally matured endosperm and embryos when the color of the seed coats was bleached out with Eau de Javelle and some of which germinated normally and have produced normal seedlings. Six seedlings are at present of fair size from 18 seeds germinated.

7. A small portion of the seed from the parthenocarpic fruits was neither parthenogenetic nor phenospermic, but contained traces of endosperm only.

8. Mrs. R. H. Thomas' results are thus partially confirmed in so far as the *N. Tabacum* variety employed in her experiments is concerned. All evidence at present available seems to show that parthenogenesis in *Nicotiana* is limited to this one strain of *N. Tabacum*—i.e., "*Nic. tabaccum* Cuba."

Transmitted March 19, 1915.

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EXPLANATION OF PLATE 35

"*Nic. tabaccum* Cuba." U. C. B. G. 200/14.

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ON THE PARTIAL STERILITY OF *NICOTIANA* HYBRIDS MADE WITH *N. SYLVESTRIS* AS A PARENT. II

BY
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CONTENTS

PAGE
Introduction 273
Response of the Hybrids and Parental Pollen to Natural and Artificial Germinating Fluids 276
Experiments upon the Relation of Nutrition to Sterility and Flower-fall 281
Summary of Results 288

I. Introduction

In 1913 one of us reported that the several F_1 hybrids made in the University of California Botanical Garden between five *Nicotiana Tabacum* varieties and *N. sylvestris* agreed with one another in producing a small quantity of open-pollinated seed, while no seed was produced on the same plants under bag (Goodspeed, 1913). These interspecific hybrids had previously been considered to be completely sterile with the exception of such evidence as is given by the experiments of Bellair (1913), who grew an F_2 from seed of apparently unprotected flowers produced on F_1 hybrids of probably much the same parentage as those with which we have been concerned. Since the publication of the original description of the partially sterile nature of these F_1 hybrids further investigations, consisting primarily of efforts to determine and modify the nature and causes of this sterility, have been continuously in progress. The present paper is the first report of the results of these investigations.

The general problem as it presents itself to us seems a complicated one, and thus it may be desirable to state it in the most general terms

before taking up the particular phase of the problem which is the subject of this report. The various F_1 hybrids between *N. Tabacum* varieties and *N. sylvestris* are replicas on a large scale of the *N. Tabacum* parent concerned in the particular cross. The dominance of the many distinct vegetative and floral characters which are summed up in the general appearance of the *N. Tabacum* parent is, for each character, practically complete, while the dominant nature of any given parental character is made more striking through the general increase in expression due to heterosis. The F_1 species hybrids are, then, distinguished from the *N. Tabacum* parent almost solely in an increased expression of all characters peculiar to it and also by the possession of a short-lived perennial habit which is characteristic of the *N. sylvestris* parent. Their flowers are normal in every respect, with the important exception that the open anthers contain a relatively small amount of light, dry, almost entirely functionless pollen as contrasted with the mass of heavy, more or less sticky pollen which is produced by the flowers of their parents. Almost every grain of the parental pollen will germinate in its own stigmatic fluid. Examination of the F_1 hybrid pollen made at the start of the experiment and often repeated thereafter showed that a very small percentage of what appeared to be normally matured grains occurs in the mass of shriveled, degenerated and undeveloped grains (cf. plate 36). These latter correspond in appearance to those which have often been figured as characteristic of the pollen of sterile plants.

One of the most striking peculiarities of the hybrid flowers is their tendency to fall soon after anthesis. A flower under bag on a hybrid plant will, in general, fall at approximately the same time that a protected, castrated flower on one of the parent plants will fall. The only capsules remaining at the end of the season, and they are relatively very few in number, contain a little viable seed. Similarly, if the pollen of the corresponding parents is used to pollinate the F_1 flowers the latter usually persist and in all such cases a little viable seed is formed. Again, in the case of a number of other attempted interspecific crosses in *Nicotiana* we have found that the foreign pollen, although in some cases it actually germinates in the stigmatic fluid of the female parent of the attempted cross, will not inhibit the falling of the castrated flower. Lastly, the case of the F_1 hybrids between *N. sylvestris* and *N. Tabacum* "Cuba" deserves mention in this connection. *N. Tabacum* "Cuba" has been shown to exhibit a rather striking parthenocarpy, holding its flowers after castration and

maturing normal fruits and parthenogenetic or phenospermic seeds (Goodspeed, 1915). The F_1 hybrid between this *N. Tabacum* variety and *N. sylvestris* makes apparently no viable seed under bag and its flowers produce no functional pollen; thus corresponding to all the other species hybrids involving *N. sylvestris* as a parent. Despite the absence of pollination and fertilization, however, all the flowers remain attached to the plant and mature normal fruits containing phenospermic seeds.

To sum up what precedes, the F_1 hybrids produce no good pollen or so very little that the chance of a normally matured pollen-grain fertilizing one of the few normally matured ovules is small. A few good ovules are matured and after fertilization with normal foreign or parental pollen yield fruits and viable seeds. Failing pollination with functional pollen and fertilization of the few good ovules, the hybrid flowers fall after anthesis. The parthenocarpic tendency of *N. Tabacum* "Cuba" is dominant and appears intensified in F_1 of the hybrid with *N. sylvestris*.

The above is a brief statement of what seems a rather complicated situation. It includes the various phases of what constitutes the general problem of partial sterility in the F_1 species hybrids of *Nicotiana*. We have chosen to attack this general problem under the following headings.

(1) A cytological problem to be concerned with (*a*) the nature and condition of the maternal and paternal chromatin in somatic mitoses, as contrasted with that of the hybrids, and (*b*) an examination of the maturation divisions of the sex cells in the hybrid with a view to determining, first, the stage at which the degeneration of the majority of the reproductive tissues takes place and, second, the chromatic condition of the nuclei in those embryo-sacs which are capable of fertilization.

(2) A general breeding problem involving a genetic analysis of the generations which can be grown from (*a*) the open pollinated seed of the F_1 hybrids and (*b*) the seed resulting from crossing the parents back on the hybrids.

(3) A histological investigation dealing with the origin and nature of the cutting-off or abscis-layer responsible for the falling of unpollinated and unfertilized tobacco flowers.

(4) A general physiological problem which up to the present time has been taken up under the following somewhat unrelated headings: (*a*) comparison of the rate of growth of parents and hybrids; (*b*)

investigation of the response of the hybrid and parental pollen to various natural and artificial germinating fluids; and (*c*) a nutrition experiment carried on in the hope that the formation of the abscisslayer might be inhibited or retarded to favor the production of a larger proportion of normal pollen.

So far as possible we have attempted to attack simultaneously all these four main aspects of the general problem. The present paper aims to report such progress as has been made with reference to certain of the points mentioned in (4) above. The various points of attack enumerated above are by no means assumed to be the only ones or the only important ones; they simply represent those which at present our facilities and the time at our disposal will permit us to undertake.

II. Response of the Hybrids and Parental Pollen to Natural and Artificial Germinating Fluids

As mentioned above, the pollen of the F_1 species hybrids contains a few grains normal in appearance among a great mass of abnormal, evidently functionless grains. We have made hundreds of attempts to secure self-fertilized seed. Protected flowers in numbers or trimmed to a single bud have been allowed to self-pollinate. Great numbers of protected flowers have by hand been close-pollinated or pollinated from other flowers on the same plant or from protected flowers on other plants in the same row. Pollen has been collected and put on the stigmas in great amount, either as a single application or in successive applications. A variety of experiments in the field have been attempted in the effort to vary the moisture conditions and the temperature surrounding individual flowers at and immediately following pollination. Not a single seed has resulted from any of these efforts and rarely has a shrunken, empty seed-capsule matured.

As has been said, only a few ovules are capable of fertilization and the production of viable seeds. In spite of the numerous efforts mentioned above we have been unable to cause the few apparently normal pollen-grains to fertilize them. This was assumed to be due either to the slight probability that the pollen tube of a chance pollen-grain would come in contact with one of the few normally matured ovules, or to the fact that such pollen-grains were unable to germinate in their own stigmatic fluid, or lastly to structural or physiological blocks to the proper penetration of the stylar tissue by the pollen tube. The presumption stood in favor of one of the latter two alterna-

tives, since every effort was made to supply abnormally large quantities of pollen on the stigmatic surfaces in hand pollinations. Pending the results of the pollen germination studies we did not attempt to isolate numbers of the grains of normal appearance for pollination. It might here be mentioned that the parent species have throughout exhibited a rather remarkable tendency to mature viable seeds under unfavorable conditions accompanying pollination. Thus, in self or cross-pollination old pollen, overmature pistils and to some extent premature pollination (cf. Hartley, 1902) seem to have relatively little influence upon the seeding qualities of a flower of the parents.

The various facts mentioned led to the experiments which are described in what follows. Of the various F_1 hybrids between *N. Tabacum* varieties and *N. sylvestris* which we have had under cultivation, only that one involving *N. Tab.* var. *macrophylla* (female parent) will be considered in this report. The F_1 hybrid *N. Tab.* var. *macrophylla* $\times$ *N. sylvestris* is known in our cultures as F_1H38, *N. Tab.* var. *macrophylla* as 22/07, and *N. sylvestris* as 69/07.

Table 1 below details the result of one of a number of similar experiments on the germination of the pollen of F_1H38 and its parents in the three natural stigmatic fluids contained in parent and hybrid flowers. The stigmatic secretions of all the various species of *Nicotiana* are variable in amount and in time of appearance in the development of the flower and respond differently to varying climatic conditions. We have found that of the F_1 hybrid and the two parent species with which we are concerned the amount of secretion appearing on the stigmatic surface is greatest in the case of *N. sylvestris*, is less in F_1H38, and is still less in the case of *N. Tab.* var. *macrophylla*. The amount that can be collected from any one of the three varies from day to day, but is always very evidently a stigmatic secretion and not a drop of condensed moisture such as can often be found in the corolla tube. The secretions were collected as drops on a cover-glass on the day of the experiment and from flowers whose anthers were about to open. In the majority of cases these flowers were under bag. A small quantity of the desired pollen was dusted into the drop of stigmatic fluid, the cover-glass inverted on a hollow-ground slide and the preparation sealed with a drop of distilled water. This hanging-drop culture was examined after lying for five hours in a moist chamber at 26° C. Germination where indicated in table 1 means the production of pollen-tubes by approximately seventy-five per cent of the pollen-grains present in the culture.

Table 1

Germination of pollen in stigmatic fluids. $F_1H38 = N.$ *Tabacum* var. *macrophylla* × *N. sylvestris*; 22/07 = *N. Tab.* var. *macrophylla*; 69/07 = *N. sylvestris*.

No.	Pollen of	Stigmatic fluid of	Germination	No Germination	Remarks
1	F_1H38	69/07	(only 7 grains)	×	Stigmatic fluid from unprotected flowers
2	F_1H38	69/07	(only 4 grains)	×	Stigmatic fluid from unprotected flowers
3	F_1H38	F_1H38		×	Stigmatic fluid from protected flowers
4	F_1H38	F_1H38		×	Stigmatic fluid from protected flowers
5	F_1H38	22/07		×	Stigmatic fluid from protected flowers
6	F_1H38	22/07	(only 1 grain)		Stigmatic fluid from unprotected flowers
7	69/07	69/07	×		Stigmatic fluid from protected flowers
8	69/07	69/07	×		Stigmatic fluid from protected flowers
9	69/07	F_1H38	×		Stigmatic fluid from protected flowers
10	69/07	F_1H38	×		Stigmatic fluid from protected flowers
11	69/07	22/07	×		Stigmatic fluid from protected flowers
12	69/07	22/07	×		Stigmatic fluid from protected flowers
13	22/07	69/07	×		Stigmatic fluid from protected flowers
14	22/07	69/07	×		Stigmatic fluid from protected flowers
15	22/07	F_1H38	×		Exceptional growth of pollen tubes
16	22/07	F_1H38		×	Cell dried out
17	22/07	22/07	×		Stigmatic fluid from protected flowers
18	22/07	22/07	×		Stigmatic fluid from protected flowers

It seems obvious from table 1 that the physiological nature of the stigmatic secretion produced in the flowers of the F_1 hybrid is not responsible for the failure of the hybrid pollen of apparently normal constitution to produce pollen-tubes. As the table shows, the pollen of both parent species germinates as rapidly and in as great amount in the stigmatic fluid of the hybrid as it does in its own. Further, the appearance of the plants from seed of unprotected flowers makes it evident that the pollen of a number of other *N. Tabacum* varieties will germinate on the stigmatic surfaces of the hybrid. None of the hybrid pollen-grains, however, will germinate in their own stigmatic fluid or in that of either parent.

Table 2, below, gives the results of one of a number of attempts to measure the reaction of the parental pollen to various artificial germinating fluids. Hanging-drop cultures of the pollen were made in tap water and in 10 per cent solutions of levulose, dextrose, and maltose. After four hours in a moist chamber at 20° C. the average length of the pollen-tubes was determined. Twenty-five measurements taken at random in the preparation gave, in each case, the average amount of growth. The length of the pollen-tubes is stated in divisions of the eyepiece micrometer with the 2/3 objective.

TABLE 2

Growth of Pollen Tubes of	In Tap Water	In 10% Levulose	In 10% Dextrose	In 10% Maltose
N. sylvestris	2.5	1.2	3.8	11.2
N. Tab. var. *macrophylla*	10.4	all burst	7.5	6.9

From the results given in table 2 it is plain that the pollen of the two species used reacts differently to the same culture medium. The pollen of *N. sylvestris* germinates rather poorly and the pollen-tubes grow slowly in tap water, as contrasted with the high percentage of germination and the rapid growth of the pollen and pollen-tubes of *N. Tab.* var. *macrophylla* in the same culture medium. Almost exactly the reverse is true of the behavior of the two types of pollen in maltose, while in levulose the pollen of *N. Tab.* var. *macrophylla* will not germinate at all and the pollen of *N. sylvestris* shows very slight germination and feeble growth of pollen-tubes. The fact that specific chemical substances appeared to exert a stimulating effect upon the germination and growth of the pollen on the parents led to the use of a variety of substances in the hope of finding one which would induce germination of the hybrid pollen of normal appearance. Numerous sugars, dilute acids and various nutritive substances in different concentrations were used as culture media. No success attended these various efforts, and it seems certain that it is not the absence of specific chemical substances in natural stigmatic secretions which accounts for the failure of the F_1 pollen normal in appearance to germinate in its own stigmatic fluid or in that of its parents.

These experiments on pollen germination as a phase of the general investigation were undertaken a number of years ago, and our attention has more recently been directed to various nutrition experiments which are to be described in what follows. In our efforts to bring about the germination of the apparently normal pollen of the F_1 hybrid we were guided primarily by the older conception of the important

role of specific substances in determining whether or not pollen will germinate. Latterly the views of Molisch and Burch emphasizing the importance of specific substances are being replaced in the light of results obtained by Martin, Tokugawa and in the later work of Jost, which indicate that general physical conditions are the determining factors in pollen germination. Complete reference to and review of the older and more recent literature is given by Martin (1913) and Tokugawa (1914). Especially important is the question of water-supply and its regulation. Jost has demonstrated in a rather wide range of cases that restricted water-supply is the significant factor in bringing about germination of pollen. Martin (*loc. cit.*) found that the germination of the pollen of red clover depends upon a proper water-supply; the range of variation in water-supply within which pollen would germinate was limited; indeed, conditions giving free water-supply to the stigma may lead to sterility. The results given in table 2 above add fragmentary evidence along this same line. Thus, the reaction of the pollen of *N. Tab.* var. *macrophylla* in tap water and in 10 per cent levulose is not susceptible of explanation on the basis of osmotic pressure, but is intelligible with reference to the effect of reagents upon the swelling of cell colloids. Inhibition of water absorption could be brought about by the calcium salts of tap water, a result in keeping with the general action of the alkali earths in such connections (Cranner, 1914, p. 536). This general tendency to assign greater importance to general physical conditions has, of course, been increasingly apparent in the work being done on seed germination, in which field the range of experiments has been far more extensive and the results apparently conclusive.

The complete failure of numerous efforts to bring about the germination of the apparently good hybrid pollen made it seem best to await the results of cytological studies on its condition before attempting further germination experiments along the newer lines indicated above. It now seems possible that the relatively few well-rounded pollen-grains of the hybrid which appear to be normally rich in cytoplasmic and nuclear substance are of abnormal constitution with respect to chromatic content. In the preparations which we have obtained showing the maturation divisions in the pollen mother-cells of the F_1 hybrid, the chromatin appears greatly fragmented or chromosome distribution is incomplete and chromosome fragments or lagging chromosomes are seen in the equatorial zone or on the spindle in telophase of the heterotypic division. It is possible that even with

abnormal nuclear constitution germination of the pollen-grains that mature can be brought about. To this end it may be necessary to supply some external conditions which are absent, such as oxygen or water-supply. Mechanical resistance to enlargement may enter in as a factor and it is possible that the histology and microchemistry of the pollen coats may give important evidence concerning the failure of the hybrid pollen to germinate. We are indebted to Dr. William Crocker of the University of Chicago for his interest in the work and for a number of suggestions which have been embodied in the above discussion.

In summing up the experiments on pollen germination detailed above we are concerned almost entirely with negative results so far as the primary objective of the experiments is concerned. It has been shown, however, that the stigmatic secretion produced in the flowers of the F_1 hybrid is a favorable medium for the germination of tobacco pollen, the pollen of normal appearance shed in these same hybrid flowers being the only exception to this statement which we have found. Further it has been shown that the pollen of *N. Tabacum* var. *macrophylla* differs from the pollen of *N. sylvestris* with respect to growth response under the influence of 10 per cent solutions of dextrose, levulose, and maltose and in tap water. At the time this seemed to indicate that these or other specific substances might be required as a growth stimulus for the germination of the hybrid pollen. Nothing was discovered, however, which would provide this stimulus and bring about germination.

III. Experiments upon the Relation of Nutrition to Sterility and Flower-fall

Our purpose in the experiments detailed below was to bring about such a condition of nutritive balance within the partially sterile F_1 hybrids under discussion that a greater proportion of normally constituted pollen-grains and ovules would be matured. As has been noted above, the flowers in the F_1 hybrids fall a short time after anthesis. This falling of the flower is due to the presence in the pedicel of an abscess-layer which is formed when pollination and fertilization do not take place. Castration of self-fertile species similarly results in the fall of the flower after anthesis while, at least in the hybrid, the formation of an abscess-layer is inhibited by the fertilization of a relatively few ovules. The partially sterile character

of the F_1 hybrids is thus particularly characterized by the early falling of their flowers, and we attempted first to inhibit flower-fall in the expectation that such inhibition might be accompanied by the production of a greater proportion of normally matured ovules and pollen grains. It is, however, obvious that we might have succeeded in bringing about an induced parthenocarpy in which the F_1 hybrids would have held their flowers after anthesis irrespective of the production of normal pollen and ovules and the accompanying pollination and fertilizations. Particular emphasis also was laid upon eliminating flower and fruit-fall since the subject is of special importance in many species cultivated commercially. With particular reference to the nutrition factor in this connection it appears to have become almost a dictum by gardeners that the tomato will drop its flowers and young fruits when grown in soil over-rich in barnyard manure, while many orchard fruits fall at different periods of development without specific cause so far as determined. The problem of flower and fruit-fall has not, so far as we have been able to discover, been investigated from the strictly physiological point of view, the only type of investigation that would seem to promise valuable results. We have, in particular, been unable to find notice of experiments similar to ours in which an effort was made to eliminate flower-fall by varying the total and relative concentration of the available mineral nutrients (cf. Lloyd, 1914).

These experiments have extended over some three years. During the first two years interest centered upon the effect of the individual mineral nutrients, while by the experiments of this last year the effect of varying the total concentration of nutrient salts was sought. Rooted cuttings F_1H38 were employed during the first year and thereafter seedlings of the same hybrid a month old or less. The plants, in six-inch pots, heavily paraffined, were grown to maturity under glass. The temperature about the plants was rather highly variable but moisture conditions were maintained fairly constant.

Table 3 has to do with the experiments of the second year. Forty-eight seedlings were planted in pots each of which contained 1720 g. of washed sand. The plants were divided into three groups which are mentioned in table 3 as groups A, B, and C. Each group consisted of eight plants, with a duplicate for each of the eight which received identical treatment in each case. In group A of table 3 nitrogen was the only varying factor and the other salts remained constant in amount within the group. Similarly in group B phos-

TABLE 3

	Nitrogen (grams of sodium nitrate)	Phosphorus (grams of mono-calcium phosphate)	Potassium (grams of potassium sulphate)	Magnesium (grams of magnesium sulphate)	Total concentration, grams
GROUP A					
Pot 1	.017	1.5	3.0	1.2	5.72
Pot 2	.086	1.5	3.0	1.2	5.79
Pot 3	.172	1.5	3.0	1.2	5.81
Pot 4	.430	1.5	3.0	1.2	6.13
Pot 5	.860	1.5	3.0	1.2	6.56
Pot 6	1.290	1.5	3.0	1.2	6.99
Pot 7	1.720	1.5	3.0	1.2	7.42
Pot 8	2.580	1.5	3.0	1.2	8.28
GROUP B					
Pot 1	4.0	.086	3.0	1.2	8.29
Pot 2	4.0	.344	3.0	1.2	8.54
Pot 3	4.0	.688	3.0	1.2	8.89
Pot 4	4.0	1.376	3.0	1.2	9.58
Pot 5	4.0	1.720	3.0	1.2	9.92
Pot 6	4.0	2.408	3.0	1.2	10.61
Pot 7	4.0	2.752	3.0	1.2	10.95
Pot 8	4.0	3.096	3.0	1.2	11.30
GROUP C					
Pot 1	4.0	1.5	.086	1.2	6.79
Pot 2	4.0	1.5	.344	1.2	7.04
Pot 3	4.0	1.5	.688	1.2	7.39
Pot 4	4.0	1.5	1.376	1.2	8.08
Pot 5	4.0	1.5	1.720	1.2	8.42
Pot 6	4.0	1.5	2.408	1.2	9.11
Pot 7	4.0	1.5	2.752	1.2	9.45
Pot 8	4.0	1.5	3.096	1.2	9.80

TABLE 4

SERIES I

	Nitrogen (grams of sodium nitrate)	Phosphorus (grams of mono-calcium phosphate)	Potassium (grams of potassium sulphate)	Magnesium (grams of magnesium sulphate)	Total concentration, grams
GROUP A					
Pot 1	.02	1.2	2.4	.96	4.58
Pot 2	.20	1.2	2.4	.96	4.76
Pot 3	1.00	1.2	2.4	.96	5.56
Pot 4	2.00	1.2	2.4	.96	6.56
Pot 5	3.00	1.2	2.4	.96	7.56
GROUP B					
Pot 1	.02	.8	1.6	.64	3.06
Pot 2	.20	.8	1.6	.64	3.24
Pot 3	1.00	.8	1.6	.64	4.04
Pot 4	2.00	.8	1.6	.64	5.04
Pot 5	3.00	.8	1.6	.64	6.04
GROUP C					
Pot 1	.02	.6	1.2	.48	2.30
Pot 2	.20	.6	1.2	.48	2.48
Pot 3	1.00	.6	1.2	.48	3.28
Pot 4	2.00	.6	1.2	.48	4.28
Pot 5	3.00	.6	1.2	.48	5.28
GROUP D					
Pot 1	.02	.3	.6	.24	1.16
Pot 2	.20	.3	.6	.24	1.34
Pot 3	1.00	.3	.6	.24	2.14
Pot 4	2.00	.3	.6	.24	3.14
Pot 5	3.00	.3	.6	.24	4.14

Series II

	Nitrogen (grams of sodium nitrate)	Phosphorus (grams of mono-calcium phosphate)	Potassium (grams of potassium sulphate)	Magnesium (grams of magnesium sulphate)	Total concentration, grams
Group A					
Pot 1	3.0	.02	2.4	.96	6.38
Pot 2	3.0	.10	2.4	.96	6.46
Pot 3	3.0	.20	2.4	.96	6.56
Pot 4	3.0	1.00	2.4	.96	7.36
Pot 5	3.0	2.00	2.4	.96	8.36
Group B					
Pot 1	2.25	.02	1.6	.64	4.51
Pot 2	2.25	.10	1.6	.64	4.59
Pot 3	2.25	.20	1.6	.64	4.69
Pot 4	2.25	1.00	1.6	.64	5.49
Pot 5	2.25	2.00	1.6	.64	6.49
Group C					
Pot 1	1.5	.02	1.2	.48	3.20
Pot 2	1.5	.10	1.2	.48	3.28
Pot 3	1.5	.20	1.2	.48	3.38
Pot 4	1.5	1.00	1.2	.48	4.18
Pot 5	1.5	2.00	1.2	.48	5.18
Group D					
Pot 1	.75	.02	.6	.24	1.61
Pot 2	.75	.10	.6	.24	1.69
Pot 3	.75	.20	.6	.24	1.79
Pot 4	.75	1.00	.6	.24	2.59
Pot 5	.75	2.00	.6	.24	3.59

Series III

	Nitrogen (grams of sodium nitrate)	Phosphorus (grams of mono-calcium phosphate)	Potassium (grams of potassium sulphate)	Magnesium (grams of magnesium sulphate)	Total concentration, grams
Group A					
Pot 1	3.0	1.2	.02	.96	5.18
Pot 2	3.0	1.2	.10	.96	5.26
Pot 3	3.0	1.2	.20	.96	5.36
Pot 4	3.0	1.2	1.00	.96	6.16
Pot 5	3.0	1.2	2.00	.96	7.16
Group B					
Pot 1	2.25	.8	.02	.64	3.71
Pot 2	2.25	.8	.10	.64	3.79
Pot 3	2.25	.8	.20	.64	3.89
Pot 4	2.25	.8	1.00	.64	4.69
Pot 5	2.25	.8	2.00	.64	5.69
Group C					
Pot 1	1.5	.6	.02	.48	2.60
Pot 2	1.5	.6	.10	.48	2.68
Pot 3	1.5	.6	.20	.48	2.78
Pot 4	1.5	.6	1.00	.48	3.58
Pot 5	1.5	.6	2.00	.48	4.58
Group D					
Pot 1	.75	.3	.02	.24	1.31
Pot 2	.75	.3	.10	.24	1.39
Pot 3	.75	.3	.20	.24	1.49
Pot 4	.75	.3	1.00	.24	2.29
Pot 5	.75	.3	2.00	.24	3.29

phorus was the variant, as was potassium in group C. In making the applications of the nutrients the constituents of the following nutrient solution were used:

80 g. sodium nitrate in 2500 cc. water
25 g. mono-calcium phosphate in 2500 cc. water
50 g. potassium sulphate in 2500 cc. water
20 g. magnesium sulphate in 2500 cc. water

At intervals of a week varying amounts of each of the above solutions were added to each pot to give the totals for each nutrient stated in table 3. A trace of ferric chloride was added to each pot.

The experiments of the first year correspond to the above and the emphasis was similarly laid upon varying the amount of individual nutrients. The range of variation in concentrations and the number of plants employed were, however, considerably less, and a full report of the experiments of the first year would add nothing to that given in table 3.

The results obtained during the first two years indicated that variations in total concentration of available nutrients, rather than particular concentrations of individual nutrients, might be the important factor in inhibiting flower-fall. To this end the experiment detailed in table 4 was devised to give the wide range of variation in total concentration together with variation in the amount of individual nutrients. One hundred and twenty seedlings were transplanted to pots containing 2000 g. of washed sand. The salts were all added to the pots along with the sand at the start of the experiment and thereafter the plants were watered at regular intervals with distilled water.

As shown in table 4, sixty different combinations were secured and a duplicate test, as in the work of the preceding year, was carried through. These combinations were equally divided into three series—I, II and III—and each series was in turn divided into four groups. In series I, table 4, nitrogen as sodium nitrate was the only varying factor within the group, the amount of each of the other salts being held constant. Correspondingly in series II phosphorus as mono-calcium phosphate was the only variant within the group, while in series III potassium as potassium sulphate was the group variant. Within each of the four groups—A, B, C, and D of each series—the increase of the varying nutrient was the same from pot 1 to pot 5. Thus in series I, pot 1 in all four groups contained .02 g. of sodium nitrate, pot 2 contained .2 g. in all four groups, and so on. As we

pass from group A to group B in any series, however, the amount of the other salts diminishes, so that B contains two-thirds, C one-half and D one-fourth the amount of a given salt contained in the pots in group A. This arrangement gives, of primary importance, a series in which the total concentration of nutrient salts varies widely and in addition gives a distinct variation in each individual nutrient both within a group and between different groups.

The results of these various experiments are almost entirely negative so far as the effort to inhibit flower-fall completely is concerned. It was, however, established that when the total concentration of available nutrients falls below a certain point almost all the young fruits are held on the plant for a very considerable period of time in place of falling, in the majority of cases, shortly after anthesis, a condition characteristic of the flowers of sister plants under field conditions. An appreciable number of fruits on many plants came almost to maturity before falling whenever the total concentration was low. The effect of low total concentration of nutrients was also seen in connection with pollinations of hybrid stigmas with normally matured parental pollen. In the field it is difficult to make such pollinations at just the proper stage in the development of the hybrid flower and many attempts are necessary to secure even a little seed. This seems to be due to the fact that under field conditions the abscisslayer in the hybrid pedicel is formed when a certain stage of pistil development is reached without pollination and the fertilization of the few good ovules matured. Thus in the field successful pollination could be secured only when the pollen was applied to the stigma just before the opening of the anthers, the latter often taking place before the complete opening of the flower. In the case of the pot cultures of sister plants which were given a low total concentration of nutrients almost every attempted pollination was successful and to a considerable extent the stage of development of the flower was of no significance. It must be definitely noted, however, that no increase in the number of ovules capable of fertilization accompanied these more generally successful back-crosses. Further evidence in the above connection is given by the fact that plants growing for a number of years in pots of unrenewed garden soil have in successive years shown a longer and longer period of retention of flowers and fruits.

On the other hand, so far as our experiments are concerned, there is no indication that an increase or decrease in the normal concentration of any one constituent of a nutrient solution will affect flower

or fruit-fall. Especially in the case of nitrogen the results in many cases contradicted the expectation in that flowers and young fruits were retained almost as long on a plant growing in a rich nitrogen culture as on one growing in a culture of low nitrogen concentration. The same was true with regard to the other nutrients whose concentration was varied or in some cases the plants under both low and high concentration dropped their flowers at the same time.

Balls (1912, p. 68) reports that "boll-shedding" in Egyptian cotton results from the formation in the flower stalk of an abscisslayer of "extreme simplicity." The stimulus inducing its formation is, according to Balls, the result of deficient root-absorption or its equivalent. Thus a disturbance in the water content of the plant is followed by a "demolition of the delicate balance between root and shoot" to produce shedding. There seems to be grave doubt as to the correctness of Balls' interpretation of the histology of the abscisslayer in cotton and we may quote Lloyd (1914, p. 65) with reference to the significance of the water relation with regard to abscission:

> In general . . . we must conclude, in view of the effects of drought upon trees and shrubs, that there is a relation between lack of water and defoliation, but it is not possible to attribute abscission directly to a reduction of water content such as may be measured. It may, however, result indirectly by the disturbance of some other relation. . . . very slight departures from the normal condition of the environment in other regards are sufficient to cause or to hasten abscission.

Balls further states that non-fertilization is not an appreciable cause of boll-shedding in Egyptian cotton, a situation evidently occasioned by the lack of rain during the season when cotton is flowering and not to be taken to mean that fruits develop readily without fertilization. The initial stimulus for the formation of the abscisslayer which causes flower and fruit-fall in tobacco is non-fertilization. We have been able to show, however, that a lowering of the total amount of available nutrients is effective in at least retarding the formation of this abscisslayer while it has, on the other hand, been shown that a variation in the concentration of an individual nutrient has little or no effect in the same direction. All the evidence at hand seems to indicate that maintenance or disturbance of general physiological states or conditions of equilibrium is a more potent factor for initiating or inhibiting stimuli or reactions than the action of specific chemical substances. Conversely, also, many effects now assigned to the action of such specific substances are more consistently explained

as due to the modification of general physiological conditions, in many instances probably to nothing more than variations in the hydration of cell colloids.

The results of the experiments above described appeared to indicate that some physiological balance of the plant, a disturbance of which causes flower-fall, was sufficiently delicate and responsive to variations in nutrition to make of value the experiments noted below in which the parent was grafted on the hybrid and *vice versa*. A very considerable number of efforts were made to secure graft unions between F_1H38, *N. sylvestris*, and *N. Tab.* var. *macrophylla*. All attempts to graft F_1H38 and *N. sylvestris* using the former both as scion and stock have been to date unsuccessful. A number of grafts of F_1H38 and *N. Tab.* var. *macrophylla* have been successfully produced, though the operation is much more certain to succeed when the *N. Tabacum* parent is used as the scion. It was held as possible that the hybrid growing on the parent might produce a larger proportion of normally matured pollen and ovules or that the parent growing on the hybrid would show some lessening of reproductive vigor. No such result was found to occur in either case. The hybrid comes into flower when growing on the parent with as small a proportion of pollen of normal appearance as it does when growing on its own roots, while the parent growing on the hybrid produces an abundance of normal pollen and the usual proportion of viable seeds is formed. However, the hybrid flowers and young fruits were retained for a much longer period than was the case when the same plant grew on its own roots under field conditions.

IV. Summary of Results

1. The F_1 hybrids between *Nicotiana Tabacum* varieties and *N. sylvestris* produce a very small quantity of pollen of normal appearance, while practically the entire contents of the anther cells consists of shriveled, functionless grains.

2. F_1 pollen of normal appearance could not be caused to germinate in its own stigmatic secretion or in that of its parents or in any one of a great variety of artificial germinating fluids.

3. The pollen of the parents germinates readily in the stigmatic secretion of the F_1 flowers. The germination and growth reaction of the pollen of *N. Tab.* var. *macrophylla* and *N. sylvestris* in tap water and in 10 per cent solutions of dextrose, levulose and maltose were

such as to give evidence against the view that specific chemical substances play the important role in determining whether or not pollen will germinate, and the results obtained are to be explained as the effect of the reagents upon the swelling of cell colloids.

4. The formation of an abscisslayer in the pedicel of tobacco flowers is the cause of flower and fruit-fall. Non-fertilization is the stimulus which is responsible for the formation of this abscisslayer.

5. From a considerable series of pot cultures it was determined that flower and fruit-fall in tobacco could be at least retarded by lowering the total concentration of available mineral nutrients, while variations in the amount of individual constituents of a nutrient solution were without effect in this connection.

6. A few normally matured ovules capable of fertilization are produced in the F_1 flowers and a little viable seed is formed after pollination with the normal pollen of the parents. The back-crosses are difficult to make in the field, since it is hard to anticipate the activation of the abscisslayer. The plants growing under conditions of low nutrient content retain their flowers so much longer that the back-crosses were almost uniformly successful. It must be emphasized, however, that no increase in the number of ovules capable of fertilization accompanied this more favorable condition for successful back-crossing.

7. Grafts between the F_1 hybrid and its parent *N. Tab.* var. *macrophylla* produced flowers containing that proportion of pollen of normal appearance and constitution and functionless pollen which is characteristic of the same plants growing on their own roots. The retention of the flowers and fruits of the hybrid was, however, favored when it was grown on the parent as a stock.

We take pleasure in acknowledging our indebtedness to Professor C. B. Lipman for continued interest in and many suggestions concerning the experiments reported upon and in progress. We are, further, under obligations to Professor Francis E. Lloyd for many suggestions as to the nature of abscission in tobacco.

Transmitted February 3, 1916

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EXPLANATION OF PLATE 36

Fig. 1. Pollen of F_1H38 photographed after one-half hour in its own stigmatic fluid. ×150.

Fig. 2. Pollen of *Nicotiana sylvestris* photographed after ten minutes in M/10 cane sugar solution. ×150.

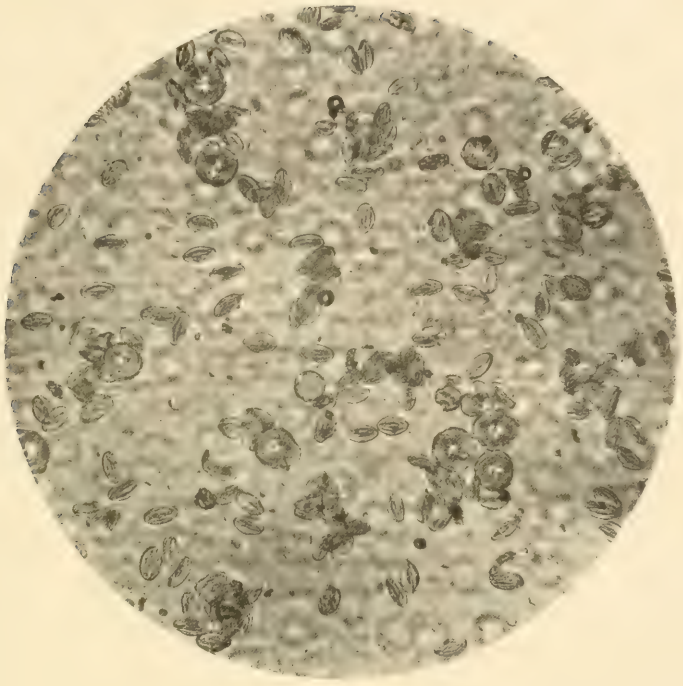

Fig. 1

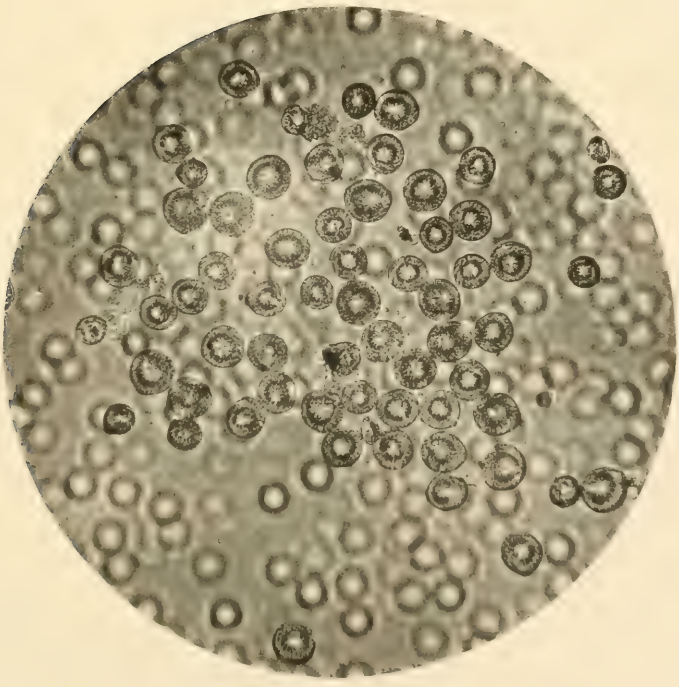

Fig. 2

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ON THE PARTIAL STERILITY OF *NICOTIANA* HYBRIDS MADE WITH *N. SYLVESTRIS* AS A PARENT. III

AN ACCOUNT OF THE MODE OF FLORAL ABSCISSION IN THE F_1 SPECIES HYBRIDS

BY

T. H. GOODSPEED AND J. N. KENDALL

As pointed out in a number of previous papers, the F_1 hybrids between *N. sylvestris* and *N. Tabacum* varieties are in practice completely self-sterile. A small proportion of the ovules borne by these hybrids are normally matured and capable of fertilization and the production of viable seeds, and while it is probable that a corresponding proportion of pollen grains are similarly capable of functioning normally their relative number is so small that attempts to secure self-pollinated seed have, up to the present time, been unsuccessful. As a result of this non-success of self-pollination all flowers under bag are abscissed at various periods following anthesis, and in the case of unprotected flowers cross-pollination resulting in the maturing of a very small quantity of viable seed is followed by the retention of less than 1 per cent of the fruits commonly held upon a plant of one of the parental species. In a previous communication a description has been given of efforts, in general unsuccessful, to inhibit this characteristic abscission of fruits, in which none of or only a very few of the ovules have been successfully fertilized, and to increase the amount of normally matured sexual elements (cf. Goodspeed and Ayres, 1916). The present paper aims to present the results of a considerable series of preliminary experiments which have indicated the mode of abscission

of flowers and fruits on the F_1 species of hybrids and have given further evidence concerning the relation between successful pollination and fertilization on the one hand and abscission of flowers and fruits on the other. The general problem of abscission in the Solanaceae with particular reference to the genus *Nicotiana* is being investigated by one of us and will be the subject of a more extended discussion later on.

We have first to consider the length of time intervening between anthesis and abscission. In the case of protected flowers of the F_1 species hybrids this period is subject to rather extensive variation (cf. Goodspeed, 1913). The following table indicates the results of a number of experiments in which tagged flowers were carefully observed from anthesis to fall. Although the hybrid flowers when protected fall as a result of unsuccessful pollination, the figures given in the following table are based upon experiments in which both hybrid and parental flowers were castrated just prior to anthesis. The influence of slight mutilations of the flowers in making the castrations is thus the same in all cases. F_1 H154 is *N. Tabacum* var. *macrophylla* (U. C. B. G. 22/07) $\times$ *N. sylvestris* (U. C. B. B. 69/07) and F_1 H179 is *N. Tabacum* "Cuba" (U. C. B. G. 200/14) $\times$ *N. sylvestris*.

Number of Days Between Anthesis and Abscission. Average	
F_1 H154	19
F_1 H179	7
22/07	6
69/07	13

(200/14; cf. Goodspeed, 1915)

The range for individual plants and their different flowers was considerably greater. For example, in the case of F_1 H154 seven different plants gave averages between 9 and 28 days, while the individual flowers on these plants showed variations in the time between anthesis and abscission of from 6 to 33 days. The statement has been made in a recent paper (Goodspeed and Clausen, 1916) that F_1 H179 holds its flowers in remarkable fashion despite the fact that its pollen is as non-functional as in the case of the other *Tabacum-sylvestris* hybrids. It may thus seem strange that we list this hybrid with the others in which abscission of almost all flowers and fruits is the rule. The point here involved demonstrates to what extent the physiological condition of the plant enters in to effect the abscission problem. F_1 H179 was first grown this past season (1915) and the plants from

seed exhibited, as noted, an unusual tendency to hold their fruits to dehiscence, so that, as they went completely out of bloom late in the fall, large numbers of capsules were found in place and filled with apparently phenospermic seed. The fact that from thousands of these seeds taken from fruits of unprotected flowers less than twenty plants have been raised demonstrates that successful cross-pollination in the field was not the cause of non-abscission. The plants of F_1 H179 from which the data given in the above table were taken came up in the spring of 1916 from their own roots, grew rapidly and flowered two months before seedling plants were set out into the field. The behavior of the flowers was watched during this period of rapid unseasonable growth. As the season advanced, more and more flowers and fruits were retained.

Despite the range of variation in abscission of flowers on individual plants and the influence of the physiological condition of the plant upon the situation in general, the above table indicates that there is a distinct difference in the length of time after castration or non-pollination during which the parent plants and their hybrids retain their flowers after anthesis.

In the second place, it is necessary to consider the length of time involved in the actual process of abscission. In this connection we are concerned with the question whether or not the abscission layer is performed in the young bud and is capable of functioning at any period of later development. This is a question which it is difficult to answer and concerning which it is not easy to obtain trustworthy evidence. Such histological and experimental evidence as is at hand seems to indicate that in the abscission of flowers and fruits of *Nicotiana* species hybrids an area of cells across the pedicel at its base is early differentiated and capable of functioning at any later time in the growth of the flower which it supports. This is indicated, in the first place, by the observable presence in young flower-buds of a zone of smaller, isodiametric cells a few millimeters up from the point of attachment of the pedicel with the main axis of inflorescence. In the second place, certain species, for example, *N. acuminata* varieties and *N. glutinosa,* shed their open flowers and all buds, even the most minute, following a sudden change in the temperature conditions to which the plant is exposed. Further, after fertilization has been successfully accomplished and the capsule and the seed begins to mature, abscission practically never takes place under corresponding environmental conditions. If a demonstrable zone of differentiated

tissue, through some part of which separation is always seen to take place, was not present from the early bud stage, or if partially matured fruits fell as readily as buds and open flowers, it might be conceivable that the formation of the abscission zone occurs only immediately following the particular "stimulus" or lack of "stimulus" upon which abscission is dependent.

Returning now to the length of time occupied by the actual process of abscission, we may note that two types of reaction were investigated. First, the abscission following non-pollination or unsuccessful pollination, and second, the abscission due to injury, "spontaneous abscission"; in this case the effect of illuminating gas in the air surrounding the plant. In the first, the tissues of the pedicels of numerous castrated flowers of all degrees of development were examined histologically to determine the time of first appearance of loosening or separation in the cells of the abscission zone. At least six and apparently not more than ten hours are necessary for the actual process of abscission caused by non-pollination or unsuccessful pollination. In the second case above the reaction is much more rapid, taking place in from one to four hours. These latter figures are based upon direct observation of the behavior of flowers on a freshly cut shoot placed in water surrounded by air into which illuminating gas had been introduced.

The results of a rather extensive histological and cytological investigation as to the position and condition of the abscission zone and the nature of the process of abscission proper are summarized below:

1. In *N. Tabacum, N. sylvestris* and the F_1 hybrids between them the abscission zone is always to be found at the base of the pedicel. This is also true of varieties of *N. Langsdorffii,* our observations on this species differing from those of Hannig (1913), who states that the abscission zone is located at the tip of the pedicel just below the calyx.

2. A conspicuous grooved ridge or ring of tissue stands out around the base of the pedicel in *Nicotiana* species. This grooved ring may be taken to indicate the position of motor tissue or merely of a node. The position of the abscission layer is independent of the groove, although the position of the general abscission zone occurs under it. The actual abscission layer ordinarily is found from five to seven rows of cells distal to the groove.

3. The abscission zone is seen in the cortex and pith to be composed of small isodiametric cells which grade up gradually to the size and

shape of the cells characteristic of these tissues in the remainder of the pedicel. The cells of the abscission zone appear to be somewhat collenchymatous and in many cases contain a greater number of starch grains than do the neighboring cells without the abscission zone. The abscission zone extends in a ring completely around the cortex and across the pith. On the outer or ventral portion of the cortex it is ordinarily composed of from fifteen to twenty rows of cells, while on the inner or dorsal side the zone is wider and spreads out into the considerable area of storage cells found in the axil of the pedicel.

4. Abscission apparently may take place in almost any portion of the abscission zone distal to the groove. In both cortex and pith the cells taking part in abscission and most distant from the vascular cylinder may be from five to ten rows in thickness, but as the vascular tissues are approached the abscission layer is gradually reduced in thickness until separation takes place along only one row of cells. In the cortex this behavior results in a wedge-shaped ring of abscission cells, the wedge being widest below the epidermis, while in the pith a circular mass of cells is involved.

5. Abscission starts in the cortical tissues just beneath the epidermis on the ventral side and extends around the cortex, taking place finally on the dorsal side. The first external indication is a bulging out of the epidermis just above the tissue in which the abscission process is taking place. This process apparently originates independently in the pith and simultaneously with the start of abscission in the cortex. There is no indication of cell division accompanying abscission in the tissues involved, nor is there evidence of any alteration of the cell walls by the dissolution of the middle lamellae or elongation and softening of the entire wall. The cells of the abscission layer become separated one from another over a considerable area or along only two rows of cells, depending, as noted above, on the proximity to the vascular tissues. This separation appears to be initiated simply by an increased turgor, as a result of which the cells round up, intercellular spaces increase in size and the contact points are successively reduced. The distention of the epidermis at the start of abscission, the entire collapse in the pith of all the cells concerned and the fact that a wilted shoot with less than the normal turgidity retains its flowers following injury but sheds them as soon as turgid—these facts seem to favor this view. This question as to the physiology and mechanics of cell separation in the abscission zone is still receiving attention; the statements made in the preceding sentences represent

the only conclusions that could be drawn from the considerable mass of data at present available.

7. At the completion of the process of abscission the flower or fruit is often retained for a short time or until mechanical agencies break the epidermis and the few tracheal elements still intact. For this reason a slight tapping of the flowers or fruits is necessary to determine exactly the time of complete separation in collecting data such as are presented in the above table. Longitudinal sections of the detached pedicel after abscission show a distinctly convex outline at the abscission surface with a slight notch at the tip. The surface itself is composed of the protruding rounded ends of cells, with here and there a spherical, completely isolated cell mechanically adhering, and broken ends of tracheal elements. The surface cells, both those in place and those isolated, are normal in every respect and apparently do not differ from the cells of the abscission layer prior to separation nor from the remainder of the cells of corresponding tissues so far as thickness of wall and size, shape, and character of cell inclusions are concerned. The exposed, basal, attached portion of the pedicel corresponds in appearance, except that the abscission surface is flat, not convex. Soon after separation the cells on this exposed surface collapse and form a comparatively heavy protective layer.

8. The number of cells actually concerned in the process of abscission is greater in the hybrids mentioned above than in the parental species. The same is true in the case of "automatic" as contrasted with "spontaneous" abscission.

The preliminary nature of this report was indicated at the start. A full discussion of the literature dealing with and bearing upon abscission, together with the results of more extensive experiments at present in progress, will be the subject of a more extended communication.

We take pleasure here in mentioning the obligation under which Professor F. E. Lloyd has placed us for his many helpful suggestions in connection with the work reported upon above.

Transmitted September 8, 1916.

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THE NATURE OF THE F_1 SPECIES HYBRIDS BETWEEN NICOTIANA SYLVESTRIS AND VARIETIES OF NICOTIANA TABACUM

WITH SPECIAL REFERENCE TO THE CONCEPTION OF REACTION SYSTEM CONTRASTS IN HEREDITY

BY

T. H. GOODSPEED AND R. E. CLAUSEN

The partially sterile F_1 hybrids between *Nicotiana sylvestris* and various varieties of *N. Tabacum* have been a subject of investigation for a number of years in the University of California Botanical Garden. As stated in a previous paper (cf. Goodspeed and Ayres, 1916), the problems which have arisen in connection with a study of these hybrids are being attacked from a number of points of view. The present report is the first which takes up definitely the data bearing on the genetic aspect of the general problem and, in this particular connection, it cannot claim to be more than introductory in nature, while it is equally concerned with providing evidence in support of a theoretical contention recently advanced (cf. Goodspeed and Clausen, 1916). In this latter discussion it was pointed out that hereditary behavior in certain cases may, for a thoroughly consistent explanation, be looked upon as a result of the interaction of distinct reaction systems rather than as a result of the expression of specific factor differences within a common Mendelian system. These two rather fundamentally different conceptions may be thought of as applicable to species hybrids and other wide crosses in the first instance and to the results of varietal crosses in the second. Thus, one

may conceive in a varietal cross that the reaction occurs between sets of factors common to a given system and that the particular type of behavior exhibited is dependent upon hardly more than a superficial difference in the case of relatively few of the factors involved. On the other hand, evidence has been obtained from the results of the *Tabacum-sylvestris* hybridizations which indicate that in such wide crosses there are concerned not contrasts between factors of a common reaction system, but fundamentally distinct reaction systems functioning to a certain extent as units in themselves. In presenting this conception these *Nicotiana* hybrids were briefly mentioned as evidence bearing out the suggestions made, and it is the purpose here to emphasize and amplify in descriptive manner the condition of affairs elsewhere only touched upon.

The data presented in what follows seem to demonstrate that when varieties of *Nicotiana Tabacum* are crossed with *N. sylvestris* the *Tabacum* reaction system dominates the course of somatogenesis nearly or quite to the exclusion of the *sylvestris* reaction system. In addition to the evidence on this point herein to be presented we have available a considerable mass of quantitative data dealing with the size and form of vegetative and floral organs (cf. Goodspeed and Clausen, 1915) which, though pertinent and bearing out our main contention, is not essential to establish the conclusion here emphasized. An attempt has been made to elaborate and at the same time cut down the amount of purely descriptive matter by the inclusion of a considerable number of photographs and drawings of the experimental material. Most of the photographs were not taken to give evidence in the present connection, and thus it has not been possible in some cases to secure illustrations of plants of parent and hybrid in exactly or even approximately the same stage of development. In the main the photographs of plants or portions of populations indicate simply the general habit characteristics of parents and hybrids, while the photographs of flowers and drawings of leaves give more specific evidence as to the relative size and form of parent and hybrid organs. All the drawings were made from fresh material. The leaves drawn in all cases represent the fourth significant leaf up from the base of the plant, and on a given plate are all drawn to the same scale.

The following hybrids in F_1 are discussed in this paper. The garden number in the University of California Botanical Garden is given in each case.

H33—N. sylvestris (U. C. B. G. 69/07 and 107/01; cf. Setchell, 1912, p. 29) × N. Tabacum var. macrophylla purpurea (U. C. B. G. 25/06, *ibid.*, p. 10).

H36—N. (Tabacum) angustifolia (U. C. B. G. 68/07, *ibid.*, p. 9) × N. sylvestris.

H38—N. Tabacum var. macrophylla (U. C. B. G. 22/07, *ibid.*, p. 8) × N. sylvestris.

H40—N. Tabacum var. calycina (U. C. B. G. 110/05, *ibid.*, p. 6) × N. sylvestris.

H142—N. Tabacum "Maryland" (U. C. B. G. 78/05, *ibid.*, p. 5) × N. sylvestris.

H179—N. Tabacum "Cuba" (U. C. B. G. 200/14, Goodspeed, 1915) × N. sylvestris.

With the exception of the last, these hybrids and their reciprocals have been remade on numerous occasions during the past five years with entirely corresponding results in every case.

A few general statements might be made concerning the above hybrids and their parents before taking up the more detailed individual descriptions. In the first place, *sylvestris* is characterized by a limited perennial habit, and plants in the garden have come up a number of years in succession from what appear to be adventitious buds produced at the base of the stem. In other words, the parts above ground die down during the winter and entirely new shoots come up from the surface of the ground in the spring. Such a limited perennial habit is unusual in the case of an herbaceous tobacco and is in general peculiar to such arborescent species as *N. tomentosa* and *N. glauca*. The *sylvestris* parent is also distinguished from the *Tabacum* varieties by its very slow rate of early growth, as a consequence of which the rosette stage of development is so long maintained that when sown late the plants in our cultures pass the entire season in rosette and do not flower until the following growing season. Finally we may note, as a character distinctly peculiar to *sylvestris*, the strikingly pendent habit of the flowers on the dense inflorescences, a habit maintained from the late bud stage up to or slightly after anthesis. This *sylvestris* character is well shown in plate 40, figure 2, and perhaps more than any other superficial character sets this species apart from all other species and from the F_1 hybrids under consideration here.

With reference to these more general characters peculiar to *sylvestris* it is to be noted that the F_1 hybrids are alike in exhibiting,

first, very rapid early growth relative to their size and, second, an ability to live over the winter season for a limited number of years. Their rate of early growth brings them to maturity at approximately the same date as their respective *Tabacum* parents, although they are always somewhat larger than and in some cases twice as large as this parent. The ability of the F_1 hybrids to flower a second and even a third season on their own roots has previously been taken to represent a tendency inherited directly from their *sylvestris* parent. It now seems probable, however, that this seeming perennial habit should be looked upon simply as a manifestation or resultant of the greatly increased vegetative vigor which they exhibit when compared with their parents. This is in part suggested by the fact that the hybrids do not die down and come up again from the base of the stem or from the roots, but the less woody portions of the laterals and main axis simply die back during the winter and masses of new laterals from dormant or adventitious buds clothe the framework of the old plant during the following growing season.

The partial sterility of these F_1 species hybrids should also be briefly mentioned here (cf. Goodspeed and Ayres, 1916). Their flowers produce only a small proportion of pollen of normal appearance and it has not been possible to cause this pollen to germinate in its own stigmatic fluid, in that of its parents, or in artificial germinating fluids. In other words, the pollen of normal appearance produced by these hybrids seems to be as strictly functionless as the evidently impotent, shrunken grains which make up the greater portion of the contents of the anthers. On the other hand, a few good ovules are produced in these same flowers and viable seed is matured when they are open-pollinated or crossed back with the pollen of the corresponding parent. However, as has been indicated, apparently no seed can be produced as a result of self-pollination, and indeed selfed F_1 flowers fall a short time after anthesis, due to a lack of successful pollination. In concluding these general remarks it might be noted that reciprocal crosses in this series of hybrids have always given results identical in every respect.

F_1H36—N. (Tabacum) angustifolia × N. sylvestris

This hybrid is figured in plates 38, 43, and 46. Plate 38 exhibits the hybrid placed between the two parents. The plant of *sylvestris* on the left is not a representative individual even so far as general habit is concerned and was taken up from the garden during the third

year of growth upon its own roots. It is also out of flower. Its height, as contrasted with that of the hybrid and of *angustifolia*, is, however, rather characteristic and in general its distinction from *angustifolia*, and thus from the F_1 also, is no more striking than is that of the thoroughly typical plant of *sylvestris* shown in plate 40, figure 2. *Nicotiana* (*Tabacum*) *angustifolia* is one of the most conspicuous members of the assemblage of *Tabacum* forms represented in our cultures. The distinctly petioled leaf is very noticeable, as is the characteristic drooping habit of the leaves. This latter peculiarity is emphasized by the occurrence of narrow, strap-like leaves along the upper third of the laterals, which leaves hang almost straight down from their point of insertion.

The F_1, as shown in plate 38, represents an expanded expression of the general *angustifolia* habit. It is to be noted in this connection that the rather marked corymbose habit of *angustifolia* might not seem to be reproduced in the F_1 since the laterals do not overtop and indeed do not equal the height of the terminal inflorescence. This situation may, it seems, be taken to represent merely a resultant of the generally increased vigor of the F_1 as compared with either parent, since particular F_1 individuals restricted in growth by cultural conditions have been seen to exhibit a more nearly corymbose habit. The number of laterals, somewhat greater on the hybrid than on *angustifolia*, has in the same fashion shown itself to be a function of the amount of vegetative vigor displayed.

Parent and hybrid flowers are shown in plate 43. In general the infundibuliform corolla of all the *Tabacum* varieties and of the F_1 species hybrids is in striking contrast to the long, slender-tubed corolla of *sylvestris* which, though slightly enlarged somewhat above the middle of the tube, narrows again as the tube passes out into the limb. Of all the *Tabacum* forms here figured, *angustifolia* is characterized by the most slender corolla tube and the least inflated infundibulum, and still its corolla tube characters and those of the F_1 hybrids set it off sharply from *sylvestris*. The calyx of *sylvestris* is also highly characteristic and is in definite contrast to the various modifications of the *Tabacum* calyx seen in its several varieties. Thus, the deeply cleft calyx tube of *angustifolia* which is clearly apparent though less ample in the F_1, is distinctly different from the smaller calyx tube of *sylvestris* with its short, blunt teeth. Finally the configuration of the corolla limb in *sylvestris* is distinct from that of any of our *Tabacum* varieties. In this case, for example, the deeply divided corolla limb,

made up of long, narrow lobes ending in slender lanceolate tips peculiar to *angustifolia* and the F_1, bears no resemblance to the corolla limb of *sylvestris,* in which the lobes are broadly triangular and the lobing rarely extends over one-third the way in from the margin. The increased size of the F_1 flower shown in the photograph, as contrasted with the flower of *angustifolia* to which it otherwise closely corresponds, may again be assigned to the greatly increased expression of all organs of the F_1 as indicated in plate 38. It is even more significant that the relation of length of tube to spread of limb in the F_1 corresponds closely to that displayed by *angustifolia* as contrasted with the corresponding relation displayed by *sylvestris.* Flower color in *angustifolia* is a very delicate shade of pink and, as contrasted with the dead white of *sylvestris,* the F_1 flower is hardly distinguishable in color from its *Tabacum* parent.

The domination in F_1H36 of the particular *Tabacum* reaction system concerned is nowhere more striking than in the case of leaf characters. This point is brought out in plate 38 and treated specifically in plate 46. The long-petioled nature of the leaves of *angustifolia* distinguishes this form sharply from the sessile-leaved *sylvestris* as well as from the other *Tabacum* varieties, practically all of which bear sessile, more or less auriculate leaves. The leaves of *angustifolia* and of F_1H36 in plate 46 were at the time selected to show the peculiar extensions of the leaf-blade which, in the case of the majority of the leaves of parent and hybrid, is decurrent along only the upper half of the petiole. The dominance in the F_1 of the leaf-complex factors introduced by *angustifolia* is in the drawing too distinct and comprehensive to require further comment here. One should note, however, the presence in the F_1 leaf of the rather long, laterally curved point characteristic of *angustifolia,* for throughout in the hybrids under discussion here the leaf-tip characters peculiar to particular *Tabacum* varieties are remarkably reproduced in addition to more general characteristics of shape and form of leaf.

F_1H38—N. Tabacum var. macrophylla × N. sylvestris

This hybrid is figured in plate 39, figure 1, in plate 44, figure 1, and in plate 47. It has throughout been one of the most striking of the species hybrids under observation in our cultures. Its *Tabacum* parent, *macrophylla,* as we have grown it, exhibits the lowest, most diminutive habit (two to three feet) of any of the *Tabacum* varieties, and thus by comparison F_1H38 (six to seven and one-half feet) is

exceedingly noticeable. The general habit of the F_1 corresponds exactly to that of *macrophylla*, not only in the relative number of laterals and the angle which they make with the main axis, but also in that this main axis in both cases is short and is overtopped by most of the larger laterals.

The correspondence of the F_1 flower and leaf characters with those of *macrophylla* as shown in plate 44, figure 1, and in plate 47, are, again, too striking to warrant further comment except with reference to certain specific points. Thus, the basal portion of the F_1 leaf compares remarkably closely with that of *macrophylla* in the broad, clasping base with rounded lobes which can hardly be called auricles. In other words, the leaf-base characters of *macrophylla* are near those of *sylvestris* in the sense that they do not include the broad, clasping auricles which are seen in most of the sessile-leaved *Tabacum* varieties and yet no influence of *sylvestris* is here apparent. The same is as strikingly true of the leaf-tip characters, since *macrophylla* bears a more bluntly tipped leaf than almost any of the *Tabacum* group and is thus very near to *sylvestris* in regard to this character.

In the case of the floral characters we may note first that the color in F_1 is nearly indistinguishable from that of the *Tabacum* parent, being in general of a deep rose-red shade. Further, the triangular, whitish areas at the bases of the sinuses are seen to correspond in amount in the two flowers and add to the general color resemblance. Finally, the nearly pentagonal outline of the limb in *macrophylla* is perfectly reproduced in the F_1 flowers. We have seen, in dealing with F_1H36, that the limb of the *angustifolia* flower is much more deeply lobed than that of *sylvestris* and here we have the *macrophylla* flower with practically no lobing, and thus representing the other extreme, yet in both cases the F_1 flowers reproduce almost exactly the shape of limb characteristic of the corresponding *Tabacum* parent.

F_1H40—N. Tabacum var. calycina × N. sylvestris

This hybrid and its parents are shown in plate 39, figure 2, and in plate 45. *Calycina* bears an unusual, rather distinctly teratological flower, types of which are illustrated in plate 45, and possesses an almost equally unusual habit in that the laterals so much overtop the terminal inflorescence that the latter is almost obscured. The flowers of *calycina* figured in plate 45, figure 2, were selected to exhibit the extreme expression of the double "hose-in-hose" character. The

flowers of the F_1 in plate 45, figure 1, were, on the other hand, picked out to show the range of expression of the calycine flower type and to demonstrate that even those flowers which on superficial examination appear to be normally developed actually exhibit a trace of the petaloid abnormality in the calyx. Thus, the calyx of the flower in the upper right-hand corner of plate 45, figure 1, has an indistinct streak of light-colored tissue at the point where the pin is seen to pass through the flower. The two flowers below show increasing amounts of this petaloid tissue. Now *calycina* bears many flowers with only this slight amount of petaloidy of the calyx and figures 1 and 2 of plate 45 might be combined to represent the total range of floral abnormality in *calycina* and in the F_1 also. However, the flowers shown in figure 1 are representative of the majority of the F_1 flowers, while those shown in figure 2 are correspondingly representative of the majority of the flowers of *calycina*. A number of varietal hybrids in which *calycina* has been crossed with other *Tabacum* varieties have been grown through many generations. In these hybrids the calycine-flower character is recessive to normal flower. In the species hybrids, on the other hand, the calycine-flower factor of the dominant *Tabacum* reaction system which is acting as a unit permits the otherwise recessive calycine-flower character to be manifested in F_1. Flower color in *calycina* and F_1 H40 is the same, and varies from light red to bright pink. The corolla limb proper is light red and this color may run in streaks down the corolla tube, while the petaloid calyx may be entirely colored or partially green. Habit and leaf characters peculiar to *calycina* are strikingly reproduced in the hybrid, as shown in plate 39, figure 2.

F_1H142—N. Tabacum "Maryland" × N. sylvestris

This hybrid and its parents are figured in plate 40, plate 41, plate 44, figure 2, and in plate 48. F_1H142 is the most vigorous of all the various hybrids herein described. It overtops both its parents as must as three feet in many cases, and is correspondingly luxuriant as to vegetative and floral characters. In general habit "Maryland" and the F_1 are remarkably similar in that each shows a pyramidal or conical shape due to the large spreading basal leaves and the rapid diminution of leaf size as one passes up the main axis. This habit is especially well seen in the row of F_1H142 in plate 40, figure 1, and also in plate 41, figure 2, whereas the single plant of "Maryland," shown in plate 41, figure 1, is overmature and the large laterals pro-

duced late in development mar the characteristic shape of the plant. The relative height of these laterals is corresponding in the hybrids when at the same stage of development.

The leaf characters of parents and hybrid are well shown in the photographs and in the drawings given on plate 48. The long, drooping leaves, rather broad in the center and tapering rapidly to either end, give to "Maryland" and to the F_1 a further distinct and characteristic appearance. The correspondence in shape between the two is emphasized in plate 48. The broad, clasping auricles of "Maryland" and the F_1 are, however, not too well figured in these drawings but stand out strikingly in the photographs of plants of parent and hybrid. The leaf-tip of "Maryland" is very characteristic in its length and tendency to curve. The tips of the lower leaves of the plants shown in plate 41, figure 1, have been broken, but the upper leaves along the lower laterals bring out the character referred to, while in the plants of the F_1 figured both lower and upper leaves show this leaf-tip peculiarity.

The floral characters of parent and hybrid are shown in plate 44, figure 2. The color of the flowers of the *Tabacum* parent and F_1 hybrid is in both cases a very light pink that corresponds closely to that of *angustifolia* and F_1H36, although there is some intangible difference in shade of color between the two varieties and their hybrids which is brought out rather sharply by a Lumière plate. The similarities in configuration of flower between the one parent and the hybrids need no comment. Attention might be called, however, to the striking likeness in calyx characters wherein size proportions are corresponding and also to the long, shapely, pointed calyx lobes present in both flowers.

F_1H179—N. Tabacum "Cuba" × N. sylvestris

This hybrid has been one of unusual interest in connection with the inheritance of the parthenocarpic tendency exhibited by its *Tabacum* parent (cf. Goodspeed, 1915). As has been mentioned above, the fact that all the *Tabacum-sylvestris* hybrids produce no good pollen results in the falling of the vast majority of their flowers, usually within a day or two following anthesis. "Cuba" has been shown to mature a fair proportion of fruits from castrated flowers, while all the other *Tabacum* varieties lose their flowers after castration about as do the F_1 hybrids which lack successful pollination. In plate 42, figure 1, is shown a plant of the F_1 hybrid made between "Cuba"

and *sylvestris*. No good pollen is produced and thus one would expect the flowers to fall just as in all these other partially sterile hybrids. Contrary to this expectation, many of the flowers on F_1H179 are retained finally to mature fruits of normal size containing masses of small-sized, functionless seed of a type which has been called "phenospermic." This retention of the fruits is shown in the photograph (plate 42, figure 1) which was taken at the end of the growing season to illustrate this point. In this connection it must be noted that, just as the calycine-flower character of *calycina* is recessive in varietal crosses and dominant in crosses with *sylvestris,* so this parthenocarpic tendency of "Cuba" appears to be recessive in a varietal cross. "Cuba" crossed with "Maryland" gave an intermediate F_1 in general appearance—self-fertile, of course—but following a very considerable number of castrations of the F_1 flowers only a very few fruits matured.

This hybrid, in addition to exhibiting the parthenocarpic tendency of "Cuba," bears out with regard to other characters also the general contention that the *Tabacum* reaction system is dominant throughout. In the case of both parent and hybrid the flower is a greenish to creamy white and the almost pentagonal corolla limb and slender tube with slightly swollen infundibulum of "Cuba" is reproduced in the F_1 flower. Leaf characters of hybrid and parent exhibit an equivalent correspondence, though the two photographs on plate 42 do not bring out this point satisfactorily since the parent individual is just coming into flower, whereas the F_1 is long past the height of its growing season. The photographs, however, leave no room for doubt as to the general similarity in leaf characters.

Of the various *Tabacum* varieties concerned in these hybrids "Cuba" is the most vigorous and possesses by far the tallest habit. The F_1 as grown was no taller and indeed did not seem to be quite as luxuriant vegetatively as "Cuba," a situation brought out in the two photographs on plate 42. This fact was probably due to a crowding of the hybrid rows in the field, since they were so close together that the lower leaves were in almost complete shade after the first few weeks of the growing season. This latter fact accounts, also, for the few laterals produced on the hybrid from the central and basal leaf axils as compared with "Cuba" and other more or less superficial differences in habit. We have chosen to discuss this "Cuba"-*sylvestris* hybrid even in the absence of as complete illustrative material as has been included in the case of the other hybrids mentioned, primarily

because it illustrates the fact that such an obviously extra-normal characteristic as parthenocarpy when combined in the *Tabacum* reaction system is manifested in the partially sterile hybrid with *sylvestris* along with all the other attributes of the reaction system in which it occurs.

F_1H33—N. sylvestris × N. Tabacum var. macrophylla purpurea

This hybrid is shown with its *Tabacum* parent in plate 37. It was made in 1909 by Professor W. A. Setchell, who grew it in 1910 and has turned over to us his notes upon its general appearance. We are greatly indebted to Professor Setchell in this regard and also for his continued interest in and many valuable suggestions concerning the progress of the experiments dealing with the *Tabacum-sylvestris* hybrids. We introduce F_1H33 into this discussion with only brief comment merely as further evidence of the dominance of a reaction system, the factors within which act together in so remarkably unified a manner when in contact with a totally different set of factors which go to make up a totally different reaction system.

The plants of F_1H33 seemed when first grown, and still are, remarkable for their vigor and vegetative luxuriance, although among the species hybrids more recently made other types of the *Tabacum* reaction system crossed with *sylvestris* have produced plants of equal size. The photographs in plate 37 emphasize the correspondence between hybrid and parent both in general habit and in particular characters. Particular leaf characters are closely similar to those of the *Tabacum* parent, but the characteristic, sharply pinched-in leaf-base of *macrophylla purpurea,* which is recessive in crosses with *Tabacum* varieties having the broad type of leaf-base, is not faithfully reproduced in the F_1 hybrid. Instead, the leaves of the F_1 hybrid exhibit a narrow form of the broad type of leaf-base, an expression which is intermediate between the broad type of *sylvestris* and the sharply pinched-in type of *macrophylla purpurea.*

Flower color in the *Tabacum* parent and in the hybrid was a deep red, a somewhat deeper shade of red than that peculiar to the flowers of *macrophylla* and F_1H38. The flower of *macrophylla purpurea* is strictly of the *macrophylla* type, but considerably larger, and the F_1 flowers are identical with the former in shape and in proportion of tube length to spread of limb, though of a somewhat larger size.

DISCUSSION OF RESULTS

The results which have been presented demonstrate that in crosses involving *sylvestris* and varieties of *Tabacum* the F_1 hybrids throughout display the characters of the particular *Tabacum* variety used in the cross, but usually on a greatly enlarged scale. The consequences of the increased growth and vigor of such hybrids must not be lost sight of in judging as to the completeness of correspondence between them and their *Tabacum* parents. This is true because such stimulation may conceivably give rise to variations in proportional effect in different characters, in such cases disturbing somewhat the ratio relations usual for the character expressions, although fundamentally the whole series of characters owes its expression entirely to the directive effect of the *Tabacum* reaction system. Specifically we have shown elsewhere (Goodspeed and Clausen, 1915) that under greenhouse conditions it is possible to modily somewhat such a comparatively constant character complex as flower size, the spread of corolla decreasing significantly when compared with that found under field conditions, the length of corolla, on the other hand, increasing somewhat. It is therefore possible that whatever slight deviations may be found in these species hybrids of *Tabacum* with *sylvestris*, when compared with their parent *Tabacum* varieties under similar conditions, may be largely dependent upon differences in the specific reactions of certain character complexes to the new set of conditions obtaining in the F_1 hybrid.

The results herein set forth are particularly striking when compared with the type of behavior exhibited by *Tabacum* in varietal crosses within the group. The *Tabacum* varieties differ strikingly in a large number of characters and when crossed give in F_1 forms which are intermediate in their characters. This intermediate condition is in part undoubtedly due to the fact that each parent contributes a scattered set of factors which display more or less complete dominance, so that a hybrid is produced the general type of which occupies a position intermediate between the two parents.[1] Much of this intermediacy, however, is due to a blending in F_1 which may be followed by definite, although often very complex, segregation in subsequent generations. Very often this segregation is so complex as to indicate the existence

[1] A number of character contrasts in *Tabacum* are known which do display nearly or quite complete dominance: viz., the petioled condition of the leaf as opposed to the non-petioled or sessile condition, the normal type of flower as opposed to the abnormal, calycine type, and the normal fruiting condition as opposed to the parthenogenetic tendency which obtains in the variety "Cuba."

of a number of factor differences even in relatively simple character contrasts such as colored as against white flowers. Blending inheritance followed by complex, intergrading segregations of this kind is characteristic particularly of the size and form relations in the various organs, as for instance in the leaves. The frequent production in such segregation of extremes lying beyond those exhibited by either parent is further evidence that this condition may be largely the expression of the inter-relations of a relatively large number of factors derived from both parents. It is all the more significant, therefore, that these partially sterile hybrids of *sylvestris* and varieties of *Tabacum* furnish such faithful reproductions of the particular *Tabacum* variety concerned in the cross, for it is hardly conceivable that all the *Tabacum* factors should be dominant to the corresponding factors in *sylvestris* in the same sense that a certain *Tabacum* factor is dominant to an allelomorph in the *Tabacum* series of factors. Thus, to take a specific instance, when a red-flowered *Tabacum, macrophylla* for example, is crossed with *sylvestris* the F_1 hybrids as grown in the field show practically the some flower color as the red-flowered *Tabacum* parent. There is perhaps a slightly lower intensity of coloration in the flowers of the F_1 hybrid which may well be dependent upon their larger size. The color changes in fading are remarkably similar in the two forms. On the other hand, when such a red-flowered *Tabacum* is crossed with a white-flowered *Tabacum,* "Cuba" for example, the flowers are strictly intermediate in color. This different behavior in the two cases might of course be attributed to factor differences in the two whites used, for as a matter of fact the white of the flower of *sylvestris* is distinctly different from the white of the flower of "Cuba." Nevertheless, when the relations shown by other character complexes, such as size and form of flower, size and form of leaf, method of branching, etc., are considered, it appears more reasonable to interpret the reproduction of the *Tabacum* flower color in the F_1 hybrid with *sylvestris* as dependent upon the directive action of the set of flower-color factors brought in by the *Tabacum* parent acting by virtue of their position in the general *Tabacum* reaction system. The F_1 hybrids, therefore, are to be considered as depending for their developmental expression upon the *Tabacum* reaction system of Mendelian factors acting as a unit in contrast to the *sylvestris* reaction system which appears to remain in a latent condition while the developmental processes are expressing themselves. The modified physiological relations which result from the presence

of the *sylvestris* set of factors are apparently responsible for the increased size and vigor of the hybrids and for slight proportional variations which may appear in the degree of expression of some of the *Tabacum* characters.

The predominating influence of *Tabacum* in determining the characters of *Tabacum-sylvestris* hybrids has also been observed by other investigators. Thus Bellair (1911) has reported that when *sylvestris* is crossed with *Tabacum* the F_1 hybrid reproduces the characters of the *Tabacum* parent, although the hybrids were larger and more floriferous than that parent and were almost completely sterile. The colored plate presented by Baur (1914) illustrates this same condition, but not in such striking fashion as we have observed it. Unfortunately this colored plate is not accompanied by any statement as to the type of *Tabacum* used in the hybrid, consequently we do not know whether the *Tabacum* variety figured is the actual parent of the F_1 hybrid of *Tabacum* $\times$ *sylvestris* illustrated in the plate. The characters of the *sylvestris* flower, however, are very faithfully portrayed. East and Hayes (1912) have also reported results of the hybridization of *sylvestris* and *Tabacum,* but almost wholly from the standpoint of the increased vegetative vigor of such hybrids. Plate VI of this article would appear, however, to bear out the contentions which have been advanced as to the directive action of the *Tabacum* reaction system. On the other hand, Brown (1912), who has made a rather complete study of a hybrid between *sylvestris* and a variety of *Tabacum* which he calls "Havana," has arrived at a different result and states as a general conclusion that neither parent exercises a uniform influence over any particular character of the hybrid or over the sum total of characters. Since this study was based upon a consideration of hybrids of only one *Tabacum* variety with *sylvestris,* it is not difficult to see how the striking correspondence between varieties of *Tabacum* and their F_1 hybrids with *sylvestris* could have escaped him. Although he finds that the hybrids in the main resemble the *Tabacum* parent, he states that in general habit and form of inflorescence they resemble *sylvestris* rather closely. These statements are not, however, very well borne out by the figures he presents, and such differences as may be noted from the *Tabacum* variety may be ascribed to other causes such as we have pointed out above.

On the basis of his study of the external characters of the parents and of the hybrid between them, Brown assigns to the *Tabacum* parents an influence of approximately two-thirds and to the *sylvestris*

parent one-third in determining the characters of the hybrid. These figures are for the most part arrived at from a consideration of measurements of nineteen arbitrarily selected characters. For any particular character a certain percentage influence is assigned to each parent and the percentage totals of the set of characters selected are supposed to represent rather accurately the relative degree of influence of each parent. This matter, however, is so largely one of arbitrary interpretation that it necessarily can possess but little value. Particularly is this true in cases in which the hybrid in general surpasses both parents. It is a well-known horticultural fact that plants in general may show marked increases in the size of vegetative and even floral organs due to stimuli furnished by favorable conditions of soil and climate. Elsewhere (Goodspeed and Clausen, 1915) we have shown that even such relatively constant characters as those which go to make up the flower complex may be influenced in definite directions by external environmental conditions. Conceivably the case may be similar here, for if the *sylvestris* elements be regarded as providing merely a stimulus for the *Tabacum* reaction system which is concerned in directing the course of somatogenesis, then the *sylvestris* elements, while in a sense the cause of the increased growth, are not a part of it. Such a conception is borne out rather strikingly by a consideration of certain of the measurements given by Brown which appear to furnish a clue to the explanation of this phenomenon. If a comparable series of measurements dealing with length of leaf, length and spread of corolla, etc., be examined we find a rather striking proportional increase over the *Tabacum* parent throughout, which is not the case when these measurements are compared with those given for *sylvestris*. When particular closely related characters are considered the evidence is even more strikingly confirmatory. The length of calyx in the hybrid is about 1.2 times that in the *Tabacum* parent, and it is 1.6 times that of the *sylvestris* parent. The corolla tube length of the hybrid is about 1.3 times that of the *Tabacum* parent, but only .75 of that of the *sylvestris* parent. Corresponding measurements which we have obtained on parents and hybrids showed practically proportional increases in corolla spread and length over the *Tabacum* parent when the spread of the *Tabacum* and *sylvestris* parents was practically the same, but the length of the corolla tube of *sylvestris* was about twice that of the *Tabacum* parent concerned. The same considerations would appear to apply to the histological features which were studied. It seems, therefore, reasonable to regard the presence of the *sylvestris*

elements as furnishing a stimulus for a general increase in size in the hybrid, but the *sylvestris* characters are not to be considered as making up any considerable proportion of this increase. This statement is not, however, to be construed as necessitating a denial of any character influence of *sylvestris* in the hybrid, but merely as emphasizing the general fact that such influence is very slight indeed.

In addition to a study of the *Tabacum-sylvestris* hybrid, Brown has made a similar study of the hybrid of *Tabacum* and *N. alata.* This hybrid has been figured by East and Hayes (1912, plate VIII) and has also been reported upon by Naudin (1863). East and Hayes, and Brown obtained hybrids which were much weaker than either parent, but Naudin found the hybrid vigorous but tardy in development. The difference may possibly be connected with the fact that Naudin used *N. macrophylla* as the *Tabacum* parent, whereas East and Hayes, and Brown used much more vigorous and robust varieties of *Tabacum.* The results, however, apparently agree in demonstrating a striking resemblance between this hybrid and the *alata* parent. Brown finds that in this case the *alata* influence is of about the same extent as that of *Tabacum* in the hybrids of *Tabacum* and *sylvestris.* Here the same criticism must apply as that given of the study of the hybrids of *Tabacum* and *sylvestris.* Apparently in this case the *alata* reaction system dominates the somatogenic processes, and the *Tabacum* instead of stimulating development definitely inhibits it. An interesting indication of the fact that *Tabacum* does, however, have some influence beyond that of inhibiting the somatogenic processes is furnished by the fact that the flowers are a very light pink, rather than white as in *alata,* indicating that although the *Tabacum* elements are for the most part incompatible with those of the *alata* system, nevertheless some of them are able to react slightly with this system and to give characters which show a slight *Tabacum* influence.

For other cases of species hybridization in *Nicotiana* (in addition to the more recent work) we must consider the investigations of the older hybridists, Kölreuter, Naudin, and Gärtner, all of whom have conducted extensive investigations of the phenomena following such hybridization. Ever since Kölreuter (1761) employed the genus *Nicotiana* in the classic experiment in which he crossed *rustica* and *paniculata,* it has been a favorite subject for investigations in the phenomena following hybridization. In spite of the extended period over which the genus has been under investigation there are still many uncorrected contradictions in the literature which deal with it. Focke

(1881) has performed a useful service by pointing out a number of these contradictions, but in many cases they have merely been pointed out, no subsequent observations having been made to establish the true state of affairs. In part these contradictions may be due to confusion in the use of scientific names; in part they apparently are due to lack of consideration of the varying genetic constitutions of the individuals employed, a natural consequence of the indefinite ideas of heredity current before the general recognition of Mendelian principles. Nevertheless some of the results appear clearly to show the same general type of behavior as is reported in this paper, one parent exerting by far the greater influence in the development of the hybrid.

As an example of conflicting observations concerning the characters of hybrids, we may take those dealing with the hybrid *rustica* × *paniculata*. Kölreuter (1761) considered that the F_1 was exactly intermediate between the two parents. Gärtner (1849, p. 253) believed that *paniculata* exerted the greater influence in the hybrid and as evidence called attention to the resemblance of "*Nicot. rustico-paniculato-paniculata* ♀ × *rustica* ♂" to *paniculata*. Obviously, however, in the light of modern conceptions this can hardly be considered proof of the predominating influence of *paniculata* in the hybrid. Focke (1881) from his own observations found the hybrids more like *rustica* and considered the effect of *paniculata* as practically confined to floral characters. Lock (1909) considered his plants of this cross distinctly nearer to *rustica*, so much so in the seedling stage that he was at one time in doubt as to whether they were actually hybrids or plants from accidental self-pollination of *rustica*. In the adult stage, however, he found them intermediate in all characters. Finally East (1915) reports that the hybrid is intermediate throughout. We must, therefore, look to the point of view of the investigators themselves as a factor in determining their judgment of resemblance to one or the other parent. This fact is undoubtedly due in part to different estimates of the comparative value of resemblances in different parts of the plant.

We may now consider a few special cases. *N. paniculata* × *N. Langsdorffii* is reported by Gärtner (pp. 260, 469) to be very nearly a complete reproduction of *Langsdorffii*. The resemblance of the hybrid to *Langsdorffii* is held to be as great as that existing between *paniculata* and plants obtained by crossing back *paniculata* × *rustica* to *paniculata* for three or four generations. Lock (1909) and East

and Hayes (1912) obtained only weak and stunted plants from this cross. Lock refrains from making any general statement concerning the resemblance of the hybrid to one or the other parent, although he apparently found a predominating influence of *paniculata* in determining flower shape and color. All observers are agreed on the total sterility of the hybrid. According to Lock, fruits were produced which contained a few seeds but these failed to germinate.

N. glauca × *N. Langsdorffii* sometimes gives hybrids, but only with great difficulty (Gärtner, p. 144). In one place (p. 222) Gärtner describes it as resembling *glauca,* but in other places (pp. 267, 402) it is listed among infertile hybrids which resemble the male parent. It is completely sterile (p. 389). Focke (1881) draws the conclusion that the hybrid resembles *glauca,* but to us it seems that Gärtner intended to convey the impression that it was more like *Langsdorffii* in its characters. In reaching this conclusion we have been guided by the fact that Gärtner's statements as to hybrid and parental resemblances on p. 222 contain so many other contradictions to subsequent statements as to make it extremely probable that they were due to a slip of the pen.

N. suaveolens × *N. Langsdorffii* is easily obtained, but absolutely infertile (Gärtner, p. 194). In spite of one contrary statement (Gärtner, p. 222), we may judge that it strongly resembles *suaveolens* in most of its characters. Gärtner states on one page (p. 258) that it resembles the maternal parent so closely that were it not for its total sterility there might be doubt as to its hybrid nature. In another place (p. 362) he states that it differs from *suaveolens* only in its sterility, in the separation of the stamen filaments from the tube of the corolla, and in the bluish color of the anthers.

N. vincaeflora × *N. Langsdorffii* is listed by Gärtner in one place (p. 222) among those patroclinous in type, but in all others places its resemblance to *vincaeflora* is emphasized. On page 258 the resemblance to *vincaeflora* is reported to be just as striking as that of *suaveolens* × *Langsdorffii* to *suaveolens.* The hybrid is totally sterile (Gärtner, p. 471).

N. glutinosa × *N. Tabacum,* according to Gärtner (p. 471), is decidedly like the male parent, but the results of Kölreuter and Naudin appear to establish an intermediate behavior for crosses between *glutinosa* and various varieties of *Tabacum.* Gärtner (p. 402) states that reciprocal crosses of *grandiflora* × *glutinosa* and *chinensis* × *glutinosa* reproduce throughout *grandiflora* and *chinensis.*

N. suaveolens × *N. macrophylla* is reported to be so much like *macrophylla* that the characters of *suaveolens* cannot be detected in the hybrid (Gärtner, p. 256). It is a vigorous form (p. 527), and possesses extraordinary powers of vegetative reproduction (p. 297). It is also notable for its prolonged period of life (p. 547).

N. quadrivalvis × *N. Tabacum* is reported to be decidedly like the male parent (Gärtner, p. 471). On page 401, Gärtner includes *macrophylla* × *quadrivalvis* among infertile hybrids resembling the female parent. Satisfactory conclusions cannot, however, be drawn from the meager statements concerning the characters of these hybrids.

N. quadrivalvis × *glutinosa* is listed by Gärtner on one page (p. 286) among patroclinous hybrids, and on the next page among those which are decidedly matroclinous. Focke (1881) has pointed out a number of inconsistencies in the descriptions of this hybrid and its reciprocal. It is apparently absolutely sterile (pp. 401, 533), although the blossoms are held for some time when pollinated with pollen from the parent forms. On page 559 its dwarf habit is described. The reciprocal cross, *glutinosa* × *quadrivalvis,* is included among those which are prevailingly patroclinous in type, and there is another note as to its patroclinous character on page 257. As with *quadrivalvis* × *glutinosa,* the blossoms are abscissed a few days after opening (p. 343). It is a dwarfed and stunted form.

N. rustica × *N. quadrivalvis* gives a hybrid resembling the male parent (Gärtner, p. 222), but not so strikingly as do some other hybrids (pp. 256, 284). It occasionally sets small, shrunken fruits (p. 367) which appear toward the end of the blooming period and contain a few seeds (p. 393). The hybrid is difficult to obtain (p. 109) and the reciprocal apparently cannot be produced.

N. quadrivalvis × *N. vincaeflora* gives a hybrid in which the type of *quadrivalvis* is distinctly discernible (Gärtner, p. 256). On page 222, *vincaeflora* × *quadrivalvis* is listed among patroclinous hybrids, and statements as to its patroclinous nature are repeated at other places (pp. 267, 286, 290, 471). The hybrid is totally sterile.

N. suaveolens × *N. glutinosa* is stated to be of the paternal type (p. 267), but it has much enlarged flowers (p. 296). The blossoms are apparently as deeply colored as those of *glutinosa* itself (p. 301), but still present differences in form and color from those of the parents (pp. 318, 641). On page 404, both this cross and its reciprocal are listed among infertile hybrids intermediate in characteristics between the two parents.

N. paniculata × *N. vincaeflora* may be produced only with great difficulty (p. 146). It is decidedly like the male parent (p. 256), so much so that the only influence of *paniculata* is to be seen in slight changes in the form and color of flowers and a slight broadening of the leaves. On page 286 this hybrid is reported to be the most striking example of hybrids resembling the male parent. It is sterile. The reciprocal cross, *vincaeflora* × *paniculata,* is listed among those infertile hybrids which resemble the maternal parent.

Gärtner appears to have been particularly impressed with the varying degree of influence of one or the other parent in the development of the hybrid, and presents the following general discussion of this problem (p. 255):

> The extent and degree of deviation of the hybrid type from the habit of the parents and their individual characters is very different with different species of a given genus. With respect to this matter Kölreuter makes the following assertion: "The greater the differences between two species, the greater must be the change which occurs in the hybrid produced by crossing them; and the less the difference between the two natural species the smaller and less noticeable will be the change which occurs when they are hybridized." These two propositions do obtain in many cases, particularly the second; but in many cases they do not, particularly in certain types where in crossing the type displays entirely different relations toward the one or the other parent form, and the characters are distributed to different parts of the hybrid in diverse degrees. Very notable in this respect is the behavior of the hybrids *Nicotiana suaveolens* and *vincaeflora;* for they in their combinations with *N. Langsdorffii* retain their own type so completely that they present differences only in the separation of the filaments from the corolla tube, the bluish color of the anthers, the greenish coloration of the corolla, and the curving of the corolla tube; in the hybrid *suaveolenti-macrophylla,* on the other hand, *suaveolens* cannot be detected and *macrophylla* predominates by far. One of the most noteworthy examples of such influence is the hybrid *Nicotiana paniculato-vincaeflora;* because *N. paniculata* is so entirely converted into the type of *vincaeflora* that only in the smaller greenish blossoms, the rounded, significantly smaller, white limb, the partial separation of the filaments from the tube, which is not crooked, in the somewhat broader leaves and in the more delicate branching is a slight difference to be noted from *vincaeflora;* for neither in the whole growth and habit of the plant nor in the general form of the leaves and their wrinkled surfaces does any notable deviation occur; whereas, on the other hand, in a combination of this species with *N. quadrivalvis* ♀ the influence of the latter in the hybrid (*N. quadrivalvi-vincaeflora*) cannot be mistaken.
>
> Less striking cases of this kind are furnished by *Nicotiana rustico-quadrivalvis, glutinoso-quadrivalvis,* in which the male type predominates, by *N. grandifloro-glutinoso, Althaea cannabino-officinalis,* in which, on the other hand, the female predominates. In most of the hybrids compounded from mixed relationships, as in *Nicotiana rustico-paniculato-angustifolia, rustico-paniculato-glutinosa,* and others, the paternal type is so exclusively predominant that these hybrids might be taken as mere varieties of the paternal parents.

The above evidence, therefore, although not above reproach, indicates clearly that hybrids between species of *Nicotiana* often do exhibit a rather close resemblance to one or the other of the parents involved, instead of displaying an intermediate behavior. The suggestion has therefore been made that in such cases distinct hereditary systems have been contrasted, one of which apparently has gained the ascendancy nearly or quite to the exclusion of the other. This behavior we have taken merely as a clue to the type of relations which exist in species crosses. It is not necessary for the sake of the argument that either species should display a predominating influence in the hybrid, but in the absence of such effects, it can readily be seen that the behavior called for upon the assumption of relations dependent upon system contrasts might be obscured beyond recognition, just as is Mendelian behavior in experiments which involve a large number of factor differences.

The data bearing upon inheritance in species hybrids of *Tabacum* and *sylvestris* are reserved for a forthcoming paper. The present paper has been designed merely to deal with the resemblances of *Tabacum-sylvestris* hybrids to the particular *Tabacum* variety used in the crosses.

SUMMARY

1. Crosses between *sylvestris* and varieties of *Tabacum* always display the characters of the particular *Tabacum* variety used in the cross, but usually on a greatly enlarged scale.

2. The predominating influence of *Tabacum* in such crosses is ascribed to the dominance of the *Tabacum* reaction system as a unit when contrasted with the hereditary reaction system of *sylvestris*.

3. An examination of the literature dealing with species hybridization shows that the case of varieties of *Tabacum* and *sylvestris* is not unique, but that in many other crosses one or the other parent exerts a more or less greatly predominating influence.

Transmitted June 12, 1916.

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PLATE 37

Fig. 1. *Nicotiana Tabacum* var. *macrophylla purpurea* (25/06).
Fig. 2. F_1H33—*N. sylvestris* × *N. Tab.* var. *macrophylla purpurea*.

Fig. 1. *Nicotiana Tabacum* var. *macrophylla purpurea* (25 06).

Fig. 2. F_1H33 *N. sylvestris* × *N. Tab.* var. *macrophylla purpurea*.

PLATE 38.

107/01—*N. sylvestris.*
F_1H36—*N.* (*Tab.*) *angustifolia* × *N. sylvestris.*
68/07—*N.* (*Tab.*) *angustifolia.*

PLATE 39.

Fig. 1. The F_1 hybrid (H38) *N. Tab.* var. *macrophylla* (22/07) × *N. sylvestris* (107/01) between its two parents.

Fig. 2. The F_1 hybrid (H40) *N. Tab.* var. *calycina* (110/05) × *N. sylvestris* between its two parents.

Fig. 1

Fig. 2

PLATE 40.

Fig. 1. Portion of a row of the plants of the F_1 hybrid H142—*N. Tab.* "Maryland" × *N. sylvestris.*

Fig. 2. Portion of a row of the plants of the hybrid H121 in F_2—F_1 H33 × *N. sylvestris.* In every respect the equivalent of *N. sylvestris.*

Fig. 1

Fig. 2

PLATE 41

Fig. 1. 78/05–*N. Tab.* "Maryland."

Fig. 2. Portion of a row of the plants of the hybrid (H142) *N. Tab.* "Maryland" × *N. sylvestris.*

Fig. 1

Fig. 2

PLATE 42

Fig. 1. The F_1 hybrid (H179) *N. Tab.* "Cuba" × *N. sylvestris.*
Fig. 2. 200/14—*N. Tab.* "Cuba"

Fig. 1

Fig. 2

PLATE 43

Flowers of *N. sylvestris* (69/07), the F_1 hybrid (H36) *N.* (*Tabacum*) *angustifolia* × *N. sylvestris*, and of *N.* (*Tab.*) *angustifolia* (68/07).

PLATE 44

Fig. 1. Flowers of *N. Tab.* var. *macrophylla* ((22/07), the F_1 hybrid (H38) *N. Tab.* var. *macrophylla* × *N. sylvestris* (69/07) and of *N. sylvestris.*

Fig. 2. Flowers of *N. Tab.* "Maryland" (78/05), the F_1 hybrid (H142) *N. Tab.* "Maryland" × *N. sylvestris,* and of *N. sylvestris* (69/07).

Fig. 1

Fig. 2

PLATE 45

Fig. 1. Flowers of *N. Tab.* var. *calycina* (110/05).

Fig. 2. Flowers of the F_1 hybrid (H40) *N. Tab.* var. *calycina* × *N. sylvestris.*

Fig. 1

Fig. 2

PLATE 46

On the left a leaf of the F_1 hybrid (H36) *N.* (*Tab.*) *angustifolia* × *N. sylvestris;* on the right a leaf of *N.* (*Tab.*) *angustifolia* (68/07).

PLATE 47

On the left a leaf of *N. sylvestris* (69/07), in the center a leaf of *N. Tab.* var. *macrophylla* (22/07), and on the right a leaf of the F_1 hybrid (H38) *N. Tab.* var. *macrophylla* × *N. sylvestris*.

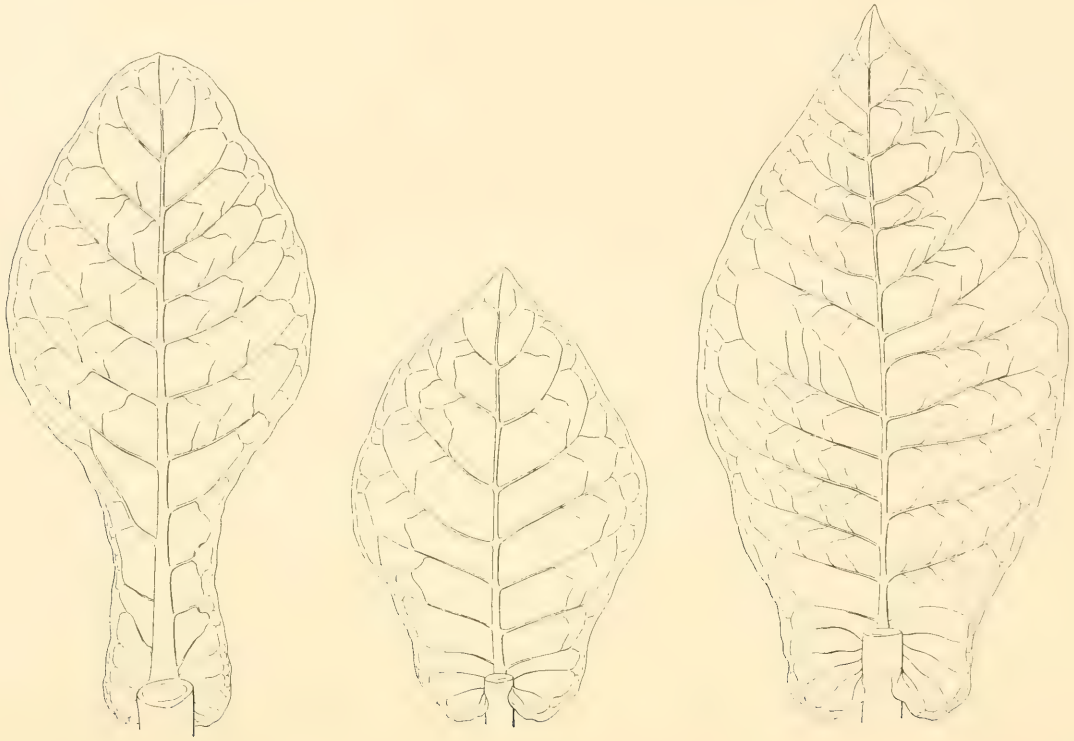
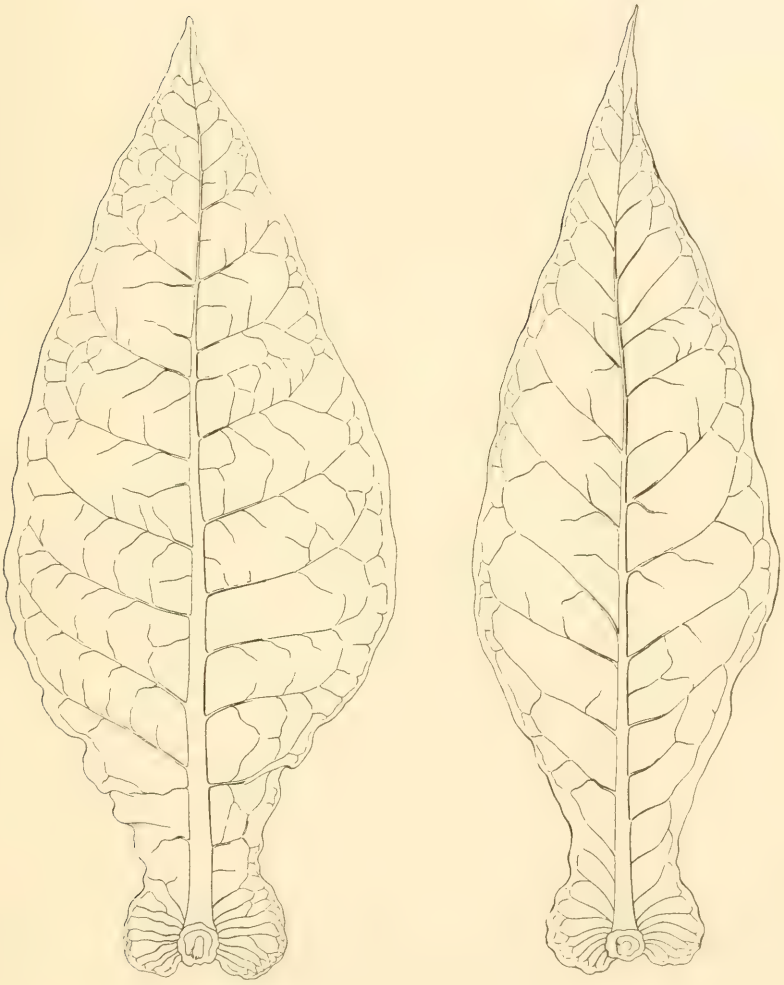


PLATE 48

On the left a leaf of the F_1 hybrid (H142) *N. Tab.* "Maryland" × *N. sylvestris;* on the right a leaf of *N. Tab.* "Maryland" (78/05).



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ABSCISSION OF FLOWERS AND FRUITS IN THE SOLANACEAE, WITH SPECIAL REFERENCE TO *NICOTIANA*

BY

JOHN N. KENDALL

CONTENTS

PAGE

I. Introduction 348
II. Summary of the literature 350
III. Technique 361
IV. Histology and cytology of the pedicel 363
1. Histological and cytological condition of the mature pedicel 363
2. Development of the separation zone in *Nicotiana* and *Lycopersicum* 367
3. Increase in size and development of mechanical tissue in the pedicel of *Nicotiana and Lycopersicum* 369
V. The process of abscission 371
1. General description of the process in several genera 371
2. Method of cell separation 376
VI. Abscission of the style and corolla 383
VII. Time of abscission 385
1. Reaction time 385
2. Abscission time 396
VIII. Experimental induction of abscission 397
1. Induction by illuminating gas 397
2. Action of acids on the separation layer of *Nicotiana* 404
3. Induction by mechanical injury 406
4. The ability of certain species to throw off pedicels from which all the floral organs have been removed, as related to the induction of abscission by mechanical injury 410
IX. Summary 411
X. Conclusion 415
XI. Literature cited 418
XII. Plates 420

INTRODUCTION

Although it is a matter of common observation that many plants are capable of detaching portions of the body, the underlying cause and the actual mechanism which bring about such separation are only slightly understood. The process has often been described as one of self-pruning by which the plant rids itself of useless portions of its body. Since abscission is sometimes confused with exfoliation, it seems desirable here to distinguish definitely between these two phenomena. It can be said that, in general, exfoliation is preceded by drying and death of the part to be cast off and that actual separation of the organ is accomplished by a mechanical break through dry, dead tissues. Abscission, on the other hand, is usually not preceded by drying and death of the organ concerned and its detachment is accomplished by a separation along the plane of the middle lamellae of active living cells.

Abscission may be either axial or lateral. Axial abscission includes the abscission of portions of stems, shoots, entire flowers or fruits. Lateral abscission includes the abscission of leaves, petioles, sepals, petals or styles. Considerable attention has been given by investigators to the abscission of flowers because of the theoretical detriment to crops caused by the fall of the flower before the fruit is formed.

The cause of leaf-fall in deciduous species is connected with periodic changes in the physiological condition brought about by changes in the environment. In the case of some herbaceous plants and occasionally in trees, sudden changes in environmental conditions resulting in a loss of physiological equilibrium often cause the throwing off of leaves, flowers or even small shoots. In certain species, anything which tends to loss or completion of function within or peculiar to an organ causes the organ to be thrown off. Thus, staminate flowers are commonly thrown off soon after anthesis and pistilate flowers generally fall when fertilization is prevented. Similarly, certain species—e.g., *Impatiens Sultani* and *Mirabilis Jalapa*—throw off portions of their stems which have been rendered useless as a part of the conducting system because of injury or removal of distal buds or leaves.

The following definitions of terms, which will be used throughout this paper, are made necessary because of a notable lack of uniformity in their usage by various investigators who have dealt with abscission.

1. *Abscission* is the detaching of an organ by the separation of actively living cells at or near its base.

2. The *separation layer* (Mohl's *Trennungschichte*) is the layer of cells the components of which will separate from one another at abscission.

3. The *separation cells* or *absciss cells* are the cells that make up the separation layer.

4. The *separation zone* is the general region through which abscission takes place and usually is largely proximal to the separation layer.

A preliminary account of abscission in F_1 species hybrids of *Nicotiana* has already appeared (Goodspeed and Kendall, 1916). The present study represents an amplification of this investigation and its extension to other species of the Solanaceae. It is particularly concerned with the following: (1) the position of the separation layer; (2) the origin of the separation layer; (3) the cytology of the separation layer; (4) the process of abscission, including (*a*) a description of the appearance of the separation layer in consecutive stages of the process and (*b*) the method of cell separation; (5) the time occupied by abscission, including (*a*) the time between the application of the stimulus and fall (reaction period) and (*b*) the time involved in the actual process of cell separation (abscission period); (6) experimental induction of abscission.

Although the investigation reported here is largely a morphological one, the results of the experiments on the method of cell separation, the time of abscission and the induction of abscission seem to have a distinct physiological significance as well.

SUMMARY OF THE LITERATURE

Since the literature on abscission is rather voluminous, it seems best to present the following discussion under several different headings corresponding, to a certain extent, with the six main topics of interest mentioned in the introduction. The summary below is largely confined to the literature on axial abscission, although that on lateral abscission is considered in so far as it has a direct bearing on the most important aspects of the abscission problem.

1. Histology of the Pedicel

a. Position of the separation layer

Hoehnel (1880), discussing the fall of catkins in *Populus* and *Salix,* locates the separation layer at the base of the catkin. The general region at the base of the catkin, in the distal part of which the separation layer is located, he calls the "separation zone." In *Salix,* actual separation occurs in the separation layer, but in *Populus* it occurs in the parenchyma entirely outside the separation layer. According to Balls (1911), the separation layer in the cotton flower is located at the base of the pedicel. The layer is located by Hannig (1913) at the base of the pedicel in *Nicotiana Tabacum, N. rustica, N. accuminata, N. sylvestris, Datura,* and *Atropa,* and at the tip of the pedicel in *Nicotiana Langsdorffii, Salvia Aloe, Cuphea,* and *Gasteria.* He finds it occurring at the middle of the pedicel in *Impatiens Sultani, Solanum tuberosum, Lycopersicum, Asparagus,* and *Begonia.* Gortner and Harris (1914) and Lloyd (1914*b*), working on the abscission of internodes as the result of injury in *Impatiens Sultani,* locate the separation layer at the first node below the injury and just above the axillary bud. Occasionally, according to the latter investigators, abscission may occur at the second or third node below the injury and in these cases the buds at the first or second nodes seem to be abortive.

The separation layer, according to Hannig (1913), may occur at the base of the complete inflorescence in *Impatiens* and *Oxybaphus.* According to Lloyd (1914*a*), the separation layer occurs at the base of the pedicel in cotton and at the base of the ripened ovary in grape "shelling." In the abscission of internodes and tendrils in *Vitis* and *Ampelopsis,* Lloyd (1914*a*) locates the layer near but not exactly at the base of the internode. A peculiar case illustrating the result of displacement of the stem on the location of the separation layer is

discussed by Lloyd (1914*a*) for *Ampelopsis* and *Gossypium*. In the latter, abscission, in the abnormal case, occurred down the internode at the base of the pedicel. This is explained as the result of a displacement during growth by which part of the pedicel becomes united to the stem.

Occasionally, grooves or swellings are noticed at the base of the organ being abscissed where they correspond more or less exactly to the general position of the separation layer. Examples are given by Hannig (1913) for *Lycopersicum* and *Solanum tuberosum* and by Balls (1911) for *Gossypium*. Abscission may occasionally occur, according to Lloyd (1914*a*), above a small bract. According to these latter investigators, there is more often no external indication of the layer. Frequently, grooves bear no relation to the layer because in many cases of this kind (Hannig, 1913, for *Brunfelsia*) separation occurs a short distance distal to the groove.

From the above brief summary it is evident that in the case of axial abscission the separation layer is located at or near the base of an internode. Apparent exceptions are reported by Hannig (1913) in which it is seemingly located at the middle of an internode. It seems probable that a more critical re-examination might reveal the fact that even these exceptions accord with the general rule. In these cases, for example, the pedicel of the flowers in question might be composed of two internodes.

b. ORIGIN OF THE SEPARATION LAYER

Kubart (1906) states that the occurrence of the separation layer in all tyes of abscission may be explained in one of the three following ways: (*a*) the separation layer is preformed and represents simply a portion of the primary meristem which has remained in its original active state; (*b*) it represents a secondary meristem; (*c*) the primary meristem may function directly as a separation layer. The difference between *a* and *c* is only a difference in time, *c* being added to explain the origin of the separation layer in abscission of very young, embryonic tissues. In *a*, the separation layer is present at the base of the organ from the start of its development, but in *b* this layer has to be formed by a secondary meristem before abscission can occur. In *a*, cell divisions are not normally found preceding abscission, but in *b* and *c* they are. Mohl (1860), working on the fall of the flower in *Aesculus, Pavia, Lagenaria, Cucumis,* and *Ricinus,* states that the separation layer in these forms is of type *b*. Throughout his entire

work Mohl gives the general impression that it is necessary for a separation layer to be formed from a secondary meristem before abscission can occur. Wiesner (1871), working on leaf-fall in general, observes that the separation layer is not generally of type *b*, as Mohl believes, but more often of type *a*. According to Becquerel (1907), the separation layer is formed in the pedicel of *Nicotiana* from a secondary meristem (type *b*). In the cotton flower Balls (1911) finds that the separation layer is of type *b*, but according to Lloyd (1914*a* and 1916*b*) there is doubt as to this conclusion, since in the case of very young cotton flowers in which abscission occurs very suddenly, he finds only rarely that cell divisions do not precede abscission. Hannig (1913), for flower-fall in general, states that a separation layer of type *a* is always present but in certain species a secondary layer of type *b* may also be formed, through which separation may or may not occur. Hannig, differing from Becquerel (1907), points out that the separation layer in *Nicotiana* is of type *a*. Lloyd (1914*a*) and Loewi (1907) indicate that in general a layer of cells through which abscission is possible is more often of type *a* than of type *b*. They believe, however, that the separation layer is not a definite morphological structure but represents merely a physiological condition.

c. CYTOLOGY OF THE SEPARATION LAYER

Mohl (1860) describes the separation cells in the flower stalk as young, active, small cells which generally contain no starch. He also states that in most cases cell divisions are characteristic of the separation layer, i.e., that the separation layer is meristematic. Hoehnel (1880) finds that cell divisions are characteristic of the proximal portion of the separation zone in *Salix* and *Populus* but in the distal portion, where the separation layer is developed, these divisions are not so numerous. In some cases he finds separation taking place in the parenchyma, entirely outside the "zone" where there had been no cell divisions. The separation cells in *Nicotiana* are described by Becquerel (1907) as small, practically undifferentiated cells with large nuclei. In *Begonia*, *Fuschia*, *Mirabilis*, and *Impatiens* Hannig (1913) describes the tissue as secondary meristem (type *b*) with the cells rectangular in shape and arranged in more or less definite rows. In contrast to the above observations, he describes the cells as small, irregularly arranged and spherical in *Salvia*, *Solanum nigrum*, and *Nicotiana Tabacum*. In *Solanum nigrum* the separation layer consists

of two or three tiers of cells but in *N. Tabacum* the layer is made up of ten to fifteen tiers.

Hannig (1913), by means of various microchemical tests, can detect no chemical difference between the cell walls of the separation layer and those of the cells on either side. Lloyd (1914*a*), however, claims that the cell walls of the separation cells break down more quickly when treated with caustic potash than do the walls of normal cells. Starch grains are frequently noted by Hannig and Lloyd (1916*a*) as occurring in the separation cells, especially in the abscission of internodes by *Mirabilis Jalapa.*

An examination of the literature thus makes it evident that there has been a great difference noted in the various species in regard to the character of the separation cells. The one characteristic of these cells, however, to which there is no exception is that they are in an actively living condition.

2. The Process of Abscission

a. methods of abscission

It has been found that in practically all cases of abscission the detaching of the organ is brought about by the separation of cells along the plane of the middle lamella. It is the method noted by Mohl (1860), Wiesner (1871), and Kubart (1906), who call it a process of maceration. Correns (1899) calls it a process of "schizolysis." Correns, however, in the same work describes a new and different method of abscission (rhexolysis) which he finds in mosses. In this latter method, separation is accomplished by a seemingly passive break of tissues irrespective of the position of cell walls. This may be the case in the style of cotton (cf. Lloyd, 1914*a*). This same method has been reported by Tison (1900) in the leaf of *Aristolochia Sipho,* although the evidence has been called in question by Lloyd and Loewi (1907). Still another type of abscission has been described by Hannig (1913) as a result of experiments on *Mirabilis* and *Oxybaphus.* In these plants he finds separation being brought about by a disorganization and dissolving away of a complete tissue. Lloyd (1916*a*), on the other hand, states that separation in these species is accomplished by cell separation and is thus true schizolysis. Hannig was doubtless confused in this case by the cell elongations which Lloyd observes and by which the membranes surrounding the protoplasts are drawn out exceedingly thin. Loewi (1907), working on

several genera, including *Cinnamomum* and *Euonymus,* notes and figures cell elongations similar to those figured by Lloyd (1916*a*). These cell elongations he finds so frequent and conspicuous that he proposes a distinct type of abscission, calling it a "Schlauchzell mechanismus."

Loewi, on the basis of his studies, seeks to classify the methods of cell separation in abscission under six different headings, which perhaps would be more appropriately presented under the next subject of consideration (the methods of cell separation); but since the author gave them as distinct methods of abscission they will be considered here. They are: (1) "round cell" mechanism; (2) dissolving of the middle lamella; (3) maceration; (4) turgescence; (5) cell elongations; (6) "hard cell" mechanism. They are to be considered merely as factors which, singly or in combinations, may enter in as a part of the normal process of cell separation. Loewi also claims that by controlling the temperature, humidity, and various other factors surrounding the plant he can influence it to such an extent as to change its method of cell separation.

b. METHOD OF CELL SEPARATION

It has been held by various investigators that the cell separation, almost universally connected with abscission, can be caused either by (*a*) chemical alteration and dissolving of the middle lamella or by (*b*) increase in cell turgor. This whole matter has received considerable attention, although very little direct evidence has been obtained. Wiesner (1871 and 1905) states that cell separation is caused by the dissolution of the middle lamella and by increased turgor. Kubart (1906) and Loewi (1907) agree entirely with Wiesner on this point. Strasburger (1913), Tison (1900), Lee (1911), Hannig (1913), and Lloyd (1916*a* and *b*) believe that cell separation is accomplished by the dissolution of the middle lamella. Practically all investigators have noticed the turgid appearance of the cells after separation, although this of course does not constitute evidence that the separation is due to increased turgor. Fitting (1911) claims that the separation is accomplished, at least in some cases, solely by an increased turgor of the separation cells. He bases his claim on the fact that abscission is very often too rapid to allow time for the dissolution of the middle lamella. He also mentions the fact that the separation cells are very often small, spherical cells, the type of cell which would respond most readily by an increase in cell turgor. On account of its

rapidity and regularity of reaction, Fitting claims that abscission is a semi-tropistic phenomenon and suggests the term "*Chorismus*" to designate this type of reaction.

It has been observed by Hannig and Fitting that the presence of various narcotic vapors in the atmosphere around certain species of plants causes their flowers or merely the petals to be thrown off. Various aspects of this general problem of the reaction of plant tissues to such agencies have been investigated. It has been determined by various plant physiologists that the presence of narcotic vapors, such as illuminating or acetylene gas, in the air around certain plant tissues causes the proportion of soluble carbohydrates within their cells to increase. This increase in the amount of soluble carbohydrates would indicate an increase in cell turgor. The question at once arises, whether or not this increase in turgor can effect complete separation or maceration of cells without the occurrence of chemical alteration in the walls. Richter (1908) resting his case on experimental evidence, throws some light on this problem. Various kinds of plant tissues which he subjected to acetylene vapors broke in pieces because of the maceration and collapse of the living cells within. He finds that in the case of the cells of tissues which are commonly rich in starch inclusions, such as the fruit of the snowberry and the potato tuber, the maceration is most complete. In the potato, for example, 3 to 5 mm. of material on the surface become completely macerated after being subjected to acetylene gas. According to Richter and Grafe (1911), the proportion of sugar in starchy seedlings subjected to acetylene gas is larger than in seedlings grown under normal conditions. In seedlings from oily seeds, however, the amount of sugar is decreased and the proportion of glycerine and fatty acids increased. The conclusion is therefore drawn that the subjection of plant tissues to narcotic vapors favors the hydrolysing process in the cells involved. The work of these two investigators goes to show that narcotic vapors may cause abscission by acting in either of the most important methods suggested as responsible for cell separation; they may increase cell turgor on the one hand or favor the hydrolysis of the middle lamella on the other.

Lloyd (1916*a*) presents evidence of chemical change in the cell walls of the separation layer before abscission. These cell walls stain in the usual manner with iodine, giving a light brownish color, but as abscission commences, they give a faint blue color when stained with iodine and washed out with water. Shortly before cell separa-

tion commences, Bismark brown and Ruthenium red fail to stain the primary and secondary cellulose membranes of the separation cells, although, when abscission does not occur, the entire cell wall is stained in the normal manner. The cells when separating seem, furthermore, to be surrounded only by the thin tertiary membranes. Lloyd, in his work, figures cells in the process of separation which show the dissolution of the primary and secondary membranes of the cell wall.

Various interpretations are given to the repeatedly observed occurrence of cell divisions preceding and accompanying abscission Mohl (1860) expresses the opinion that cell divisions are generally necessary before abscission can occur. Investigators since his time have disproved the universal occurrence of cell divisions because they find more and more cases where no cell divisions occur. Lloyd (1914*a*) maintains that cell divisions are not of necessity correlated with abscission but are merely evidences of renewed growth and wound responses. As evidence he states that cell divisions are sometimes absent and sometimes present in the same species. He cites (1916*b*) the cotton plant as a typical example in which cell divisions are present in the abscission of older flowers in which the reaction to stimulus is slow. In young flowers and flower buds abscission may proceed without cell division. He further notes (1914*a*) that cell divisions sometimes precede and at other times follow abscission in a given species.

c. AGENCIES ACTIVE IN BRINGING ABOUT THE DISSOLUTION OF THE MIDDLE LAMELLA

Very few theories have been proposed to account for the dissolution of the middle lamella and practically no evidence of any kind has been submitted. Wiesner (1905) claims that in leaf-fall an organic acid, produced as a result of lessening of cell activity and stagnation of cell contents, acts on the middle lamella. His evidence for this statement has to do with obtaining acid reactions with litmus from cells at the base of the petiole during abscission. Kubart (1906) also obtains acid reactions at the base of the corolla in *Nicotiana* during abscission and, although agreeing with Wiesner that an organic acid probably causes the dissolution of the middle lamella, he also admits the possibility that an enzyme plays a part in the process. Lloyd (1916*b*) makes the statement that the dissolution of the middle lamella is a process of hydrolysis and although making no definite statement on the subject appears to take it for granted that an

enzyme of some kind is the active factor. Indeed, since all hydrolysing processes of living cells are now supposed to be due to the action of enzymes, there is no reason to suppose that the hydrolysis of the middle lamella does not conform to the general rule. For it is known that an enzyme, pectosinase, is capable of breaking down the pectose of which the middle lamella is composed. However, until more is known concerning the nature of this particular enzyme it remains impossible to get more definite evidence on this phase of the problem.

3. Abscission of the Corolla

Reiche (1885) gives an account of the fall of the corolla in a large number of species belonging to about forty-five families of the monocotyledons and dicotyledons. He finds that the corolla may be thrown off in one of three different ways: (1) by the activity of a small-celled separation layer; (2) through decay; (3) through increase in size of the ovary, thus tearing off the tissue involved at the base of the corolla. In many cases of true abscission—case 1 above—Reiche finds that the separation layer is preformed and ready to function at any moment. This represents a contradiction of Mohl's observations, according to which the fall of the corolla is usually due to the action of a separation layer formed shortly before fall. According to Reiche, the separation layer is very seldom morphologically differentiated from the neighboring tissue, but in a few cases he describes the separation layer as consisting of a layer of cells smaller than the neighboring cells on either side.

Kubart (1906), in his account of abscission of the corolla in several different species, describes and figures the process which takes place in *Nicotiana*. The separation layer in this genus he finds to be in no way morphologically differentiated, of indefinite shape, and located about 1 mm. above the base of the corolla tube. In this general region a large number of cells separate from one another, all the cells in cross-section taking part except the epidermal cells and the tracheae. Fitting (1911), in his work on the shedding of petals, describes the process of abscission in several genera, paying particular attention to *Erodium*, *Geranium*, *Linum*, *Helianthemum*, *Perlagonium*, and *Verbascum*. Separation in these cases takes place through a region of small, spherical cells rich in protoplasm. The separation layer is not sharply differentiated as compared with the tissues on either side but is located in a restricted region at the base of the petal.

He finds no cell divisions preceding or accompanying abscission. The process in premature abscission he finds differing in no way from that in normal abscission after fertilization. These conditions, he states, correspond more or less to those which he finds in the pedicel during flower-fall.

4. Time of Abscission

The time elapsing between anthesis and flower-fall in partially sterile F_1 species hybrids of *Nicotiana* and between emasculation at anthesis and fall in the case of their corresponding parents is discussed in a previous paper (Goodspeed and Kendall, 1916). It was there stated that the average time is about nineteen days in F_1 H154, seven in F_1 H179, five in *N. Tabacum* var. *macrophylla*, and thirteen in *N. sylvestris*. When we turn to the question of the reaction time in premature abscission occurring before the normal time as the result of sudden changes in external environmental conditions, we find that this subject has received only slight attention. According to Lloyd (1914*a*), the cotton "square" falls in one to twenty-two days after the weevil lays its eggs, the average time being eight days. In one experiment in which the ovary was cut transversely, Lloyd was able to cause one hundred per cent of the young bolls to fall in forty-eight hours and ninety per cent in twenty-four hours. Larger bolls take a longer time to respond to injury than do smaller ones, as a result of the development of the pedicel to a condition in which abscission meets greater resistance. Cotton "squares," he finds, take a longer time to respond than young bolls, the former shedding thirty-five to sixty per cent in thirty-six hours and the latter forty to seventy per cent in forty-eight hours. On the other hand, he obtains no evidence (1916*b*) that the reaction times are any shorter in small buds than in larger ones. The reaction times in cases where the injury is performed in the evening seem to be shorter by about twelve hours than in cases where the injury is performed in the morning. This difference he ascribes to the increase in turgidity which takes place during the night and which serves to hasten the reaction. Very severe injuries to the ovary, he finds, cause fall of young bolls quicker than less severe injuries. Injuries which are less severe than those mentioned above and performed so as to imitate the injury inflicted on the ovary by insect larvae caused shedding in three to six days, with most of the fall occurring on the fifth day. Summing up his entire results, Lloyd

(1916*b*) states that under field conditions the responses to all kinds of stimuli conducive to abscission become evident within ten days, with the maximum frequency below six days.

The actual time involved in the process of abscission (abscission time) has received even less attention than the problems discussed above. Fitting (1911) states that abscission time may occasionally be very short, forty-five seconds to five minutes in the petals of *Verbascum* and thirty seconds to six minutes in *Geranium*. Lloyd (1914*a* and 1916*b*) finds abscission after injury of the small cotton-boll taking place within four hours, the length of time depending somewhat on the age of the boll. In a previous paper (Goodspeed and Kendall, 1916) a general estimate of the abscission time was given and it was stated that normal abscission due to lack of fertilization takes place in *Nicotiana* hybrids in four to eight hours and premature abscission in one to four hours.

5. Experimental Induction of Abscission

According to Hannig and Loewi, abscission may be induced in two different ways. First by abnormal external conditions ("spontaneous" or premature abscission) and second by normal internal conditions at the normal time ("automatic" or normal abscission). We shall consider in the following summary of the literature only two aspects of induction of the first type.

a. Induction by narcotic vapors

Hannig (1913) reports a comparative study of the behavior of cut sprigs of different species of plants when subjected to laboratory air and to illuminating gas. He notes the fact that under either of the above conditions all the flowers and occasionally a few small shoots are abscissed. He finds, however, that not all the species in a given family behave similarly in response to these conditions. We are particularly interested in the Solanaceae and we may note that this family contained more species that detached their flowers in illuminating gas than any other of the families investigated by Hannig. According to Fitting (1911), narcotic vapors such as tobacco smoke, carbon dioxide, ether, chloroform or illuminating gas frequently cause premature abscission of the corolla. He notices, however, that ammonia or turpentine vapors fail to cause abscission. Brown and Escomb (1902) make the statement that *Nicotiana*, *Cucurbita*, and *Fuchsia* shed flowers and buds in air containing only 0.114 per cent carbon dioxide.

b. INDUCTION BY MECHANICAL INJURY

Becquerel (1907), in a brief paper on the effect of wounding flowers of *Nicotiana,* notes that even after fifteen days flowers without sepals, anthers, or stigmas do not fall. After the same length of time, flowers without corollas or flowers in which the corolla or stamens are only half removed, have fallen. He points out that this result is more conspicuous in young flowers but did not investigate this point sufficiently to arrive at any definite conclusions. According to Hannig, removal of various organs of flowers frequently causes abscission but wounding of the pedicel does not. He concludes, therefore, that injury itself does not cause abscission but only acts indirectly by interfering with important physiological processes in the treated tissues.

According to Lloyd (1914*a*), shedding of very young cotton-bolls can be induced by removal of the styles before pollination, but fall in this case can be assigned, as Fitting has shown, to lack of fertilization. It appears that in the cotton flower (Lloyd, 1916*b*) there is an inhibition period which starts with the opening of the corolla and during which premature abscission as the result of sudden stimuli very seldom occurs. Also, cotton-bolls larger than 30 mm. in diameter are very seldom shed under any conditions. Other results obtained by Lloyd on the effect of injury on the abscission of cotton flowers are discussed above under "Time of Abscission" (page 357). Lloyd (1914*b*) also notes the effect of injury on abscission of internodes in *Impatiens Sultani.* Plants of this species, when a cut is made across the stem, cast off the remainder of the severed internode. He gives results of experiments on the effect of different types of injury, noting that some severe injuries do not cause abscission. Gortner and Harris (1914) have obtained similar results with the same species. They find that when the cut is made across the internode, very close to the separation layer, abscission usually occurs, but occasionally it does not. They state, as does Lloyd, that the shape and location of the separation layer may vary slightly according to the type of injury.

c. THE DIRECT OR INDIRECT ACTION OF THE EXTERNAL STIMULUS

In all the above investigations the question naturally arises, whether the narcotic vapors and injuries or any stimulus conducive to abscission act indirectly through their influence on the physiological condition of the plant or directly, through their action on the cells of the separation zone. Most investigators, except Wiesner, ex-

press the opinion that atmospheric factors work directly in causing "spontaneous" abscission, although offering, so far as I can see, no evidence for this view. Fitting states that the external influence acts directly in most cases, but that the indirect action is apparent in forms which must build a separation layer before fall can occur. In regard to the action of injury, it seems to be the opinion of most investigators (Hannig, Bacquerel, Gortner and Harris) that the stimulus acts indirectly by interfering in some way with such important physiological processes as transpiration, respiration, or assimilation. On the other hand, if abscission is sometimes a semi-tropistic phenomenon, as Fitting has suggested, it is evident that injury may act directly in causing flower-fall.

TECHNIQUE

The results noted below were obtained largely from the examination of microscopic preparations made by the paraffin method, although this method was supplemented by free-hand sections mounted in water. In investigating the condition of the pedicel in some species (*Datura* sp., *Petunia* sp. and several species of *Nicotiana*) only free-hand sections were examined. For most microchemical studies fairly thick, free-hand sections are preferable. The material for sectioning in paraffin was killed and fixed in various concentrations of the chromo-acetic series and dehydration and infiltration were, in general, carried on very slowly. The free-hand sections were mounted in water without killing.

In cutting longitudinal sections of any kind all the pedicels were oriented so that the sections were cut parallel to the main stem of the inflorescence, in the plane formed by the pedicel and stem taken together. In studying the histology of the pedicel and the cytology of the separation layer and in studying the method of cell separation, these longitudinal sections were supplemented by cross sections in series through the base of the pedicel. It was impossible to cut very thin, longitudinal sections in paraffin without crushing or breaking the cells; most of these sections therefore were cut from 10μ to 15μ in thickness. For a similar reason, it was found necessary to cut thick sections (20μ to 25μ) of the pedicels of fruits in which mechanical tissue had developed. It was possible, however, to cut excellent paraffin sections from 5μ to 7μ in thickness in cross-section or longitudinally through the small cells of the separation zone. Since the cells of the

separation zone are very small, not much could be determined in regard to the dissolution of cell walls by means of thick, free-hand sections. The best results along this line were obtained from the thin paraffin sections of the separation zone, although in order to show the cell wall in its normal thickness it was necessary to use the free-hand sections. As a supplement to these sections, several points of interest were brought out by washing off the isolated cells from the end of freshly abscissed pedicels and mounting them for microscopic examination.

In most of the work the paraffin sections were stained in safranin and Delafield's haematoxylin. The free-hand sections were generally mounted in water and stained in iodine. In special instances other stains were used. Thus, in testing for chemical differences in the cell walls of the separation cells, several other stains, such as erythrosin, eosin, Bismark brown, gentian violet and Ruthenium red were used. It was found that for demonstrating the dissolution of cell walls aqueous methylene blue was an excellent stain to use. This stain was allowed to act overnight and the sections destained slightly in alcohol. Methylene blue was also an excellent stain for the isolated cells obtained as noted above. By fixing these cells to the slide with albumen fixative and staining with this stain, the thin membranous wall surrounding the protoplast can be distinctly seen.

Various methods, such as subjecting inflorescences to illuminating gas and mechanical injury, were used to bring about abscission. The best results were obtained in cases where abscission was induced by inserting shoots under a bell-jar containing from 1.5 per cent to 3 per cent illuminating gas. By using illuminating gas in this way and by taking sections of the pedicels at intervals it was possible to determine just when the first signs of abscission appeared in a certain percentage of gas. This time was definitely determined for certain species so that it was possible to get material killed and fixed at any desired stage in the process of abscission. It was found that the best results were obtained by killing and fixing the pedicels at about the time when abscission was known to be commencing.

HISTOLOGY AND CYTOLOGY OF THE PEDICEL

1. Histological and Cytological Conditions of the Mature Pedicel

a. NICOTIANA

The vascular system in *Nicotiana*, as in all the other genera examined, is characterized by intraxylary phloem. *Nicotiana* differs slightly from all others in that the xylem seems in cross-section to be composed of a continuous ring of radial strands of tracheæ rather than composed of a broken ring of distinct bundles. When a branch of the vascular system (fig. 1, *a*) containing twenty to thirty xylem strands is given off to the pedicel, it assumes the shape of a crescent in cross-section, with the opening of the crescent on the ventral side. A short distance distal to the groove which marks the separation zone (fig. 1, *b*), the crescent closes and throughout the remainder of the pedicel the vascular system forms a complete cylin-

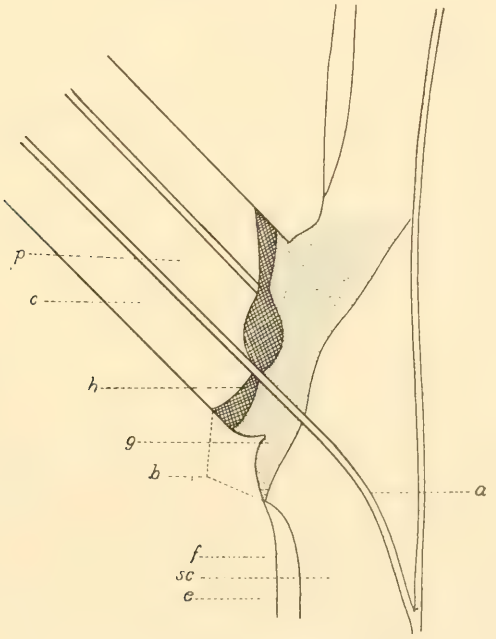

Fig. 1. Diagram of pedicel of *Nicotiana*

a—vascular system.
b—separation zone.
c—pedicel cortex.
sc—stem cortex.
e—epidermis.
f—chlorophyllous tissue.
g—groove.
h—separation layer.
p—pedicel pith.

der. The pith and cortex (fig. 1, *p* and *c*) are composed of large parenchyma cells which in the cortex are two or three times as long as wide, but in the pith are more nearly isodiametric. There is no mechanical tissue to be found in the floral pedicel but, as will be noted in more detail later, wood fibres are formed as soon as the fruit begins to develop. The epidermis of the pedicel (fig. 1, *e*) is typical but with a poorly developed cuticle, especially in the groove (fig. 1, *g*), where the cells are also much reduced longitudinally. Beneath the epidermis is a layer of small cells with very large intercellular spaces and an abundance of chloroplasts (fig. 1, *f*). This tissue stops a short distance proximal to the separation zone and does not continue in the pedicel. The layer of collenchyma which is commonly found in certain species just beneath this chlorophyl tissue is entirely absent in *Nicotiana,* or at least is very poorly developed.

Corresponding with the general region of the groove is an area of medullary and cortical cells which are smaller than corresponding cells on either the proximal or distal side of the groove. This region of small cells is homologous with the separation zone (fig. 1, *b*) and it extends across the base of the pedicel. The smallest cells are in the center of the region, in a plane with the bottom of the groove, and grade in size to the larger cells of the pith and cortex on either side (plate 49, fig. 1). The zone of small cells is ten to fifteen tiers of cells thick on the dorsal side but is wider on the ventral side, where it spreads out into the large area of storage cells found in the axil of the pedicel. The separation layer (fig. 1, *h*) is located five to seven tiers of cells distal from the bottom of the groove. Hanning reports this layer as occurring at the tip of the pedicel in *Nicotiana Langsdorffii,* but in all my experiments on two varieties of this species I find separation invariably occurring at the base of the pedicel in the position described above. All the species and varieties of *Nicotiana* examined show a structure of the pedicel corresponding with the above description except that in some varieties, as in those of *N. Bigelovii,* the separation zone is much thinner on the dorsal side. In such cases it is also noted that the groove is poorly developed.

The cells of the separation layer are in no way morphologically differentiated from those making up the remainder of the separation zone. Indeed, any cell of the zone seems capable of functioning as a separation cell. The separation cells are smaller than normal cortical cells and spherical in shape except in the vascular bundles, where they do not seem to be differentiated in size and are elongated parallel to the longitudinal axis of the pedicel. The cell walls are slightly thicker

than the walls of normal cortical cells, especially at the corners, thus giving the tissue a somewhat collenchymatous appearance. The smallest cells more proximal show this collenchymatous nature more strikingly than do the others. No difference in chemical composition could be detected, by means of microchemical tests using caustic potash, sulfuric acid, nitric acid, and various stains, between the cell walls of the separation cells and walls of other cortical cells. Other tests, however, indicated a difference in the nature of the cell contents in the two types of cells. Iodine frequently indicates the presence of starch in these cells and also colors the protoplasts a darker brown than in normal cells, showing that the separation cells are rich in protoplasm. The amount of starch in the cells, however, was found to be extremely variable, ranging from a total absence of starch to an abundance of it. Iodine green imparts to the protoplast of the separation cells a deep blue color in contrast with other cortical cells, which are not colored by this stain. The blue reaction is most prominent where the separation layer crosses the phloem. Other cells which react in the same way to this stain are the sieve tubes and companion cells and the storage cells in the axil of the pedicel.

b. LYCOPERSICUM

Conditions in *Lycopersicum* differ in certain respects from those existing in *Nicotiana.* In the former the separation zone (fig. 2, *a*) seems to be located at the middle of the pedicel and is marked externally by a swelling, as well as by the groove of the type already noted as characteristic of the pedicel of *Nicotiana.* This groove in the tomato is very deep (plate 53, fig. 1), reaching fully half the depth of the cortex, and is, furthermore, of about the same depth all the way round, differing in this respect from *Nicotiana,* where the groove is absent or poorly developed on the ventral side. The vascular system in *Lycopersicum* (fig. 2, *b*), in contrast with the condition in *Nicotiana,* is composed of scattered bundles of xylem which in this case do not form a crescent proximal to the groove but are in the form of a complete cylinder throughout the entire pedicel. Beneath the epidermis (fig.

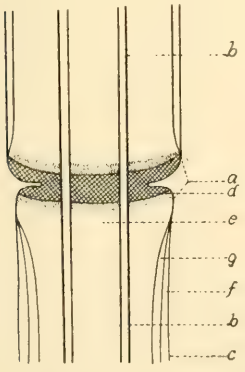

Fig. 2. Diagram of pedicel of *Lycopersicum*
a—separation zone.
b—vascular system.
c—epidermis.
d—separation layer.
e—pith.
f—chlorophyl-bearing tissue.
g—collenchyma

2, *c*) is the chlorophyl-bearing region of the cortex (fig. 2, *f*), such as occurs in *Nicotiana*, but in this case the tissue continues in the pedicel distal to the groove. Beneath this chlorophyl-bearing tissue is a layer of well-developed collenchyma (fig. 2, *g*) which however does not continue in the pedicel distal to the groove. The separation layer (fig. 2, *d*) consists of three to six tiers of cells and is located in a plane with the groove, differing in this respect from *Nicotiana*, where it is located a short distance distal to the groove. Corresponding to the condition in *Nicotiana*, the chief characteristic of the separation cells is their small size, spherical outline and active physiological condition.

c. OTHER GENERA OF THE *SOLANACEAE*

The condition of the pedicel, so far as the histology of the separation zone is concerned, was examined in several other species, a list of which is given below:

Solanum jasminioides
Solanum tuberosum
Solanum verbascifolium
Solanum umbelliferum
Solanum nigrum
Solanum marginatum
Cestrum fasciculatum
Iochroma tuberosa
Datura sanguineum
Salpichrora rhomboidea
Petunia hybrida
Salpiglossis sinuata
Lycium australis

The general condition of the pedicel of *Datura sanguineum* and *Petunia hybrida* is worth describing in some detail. The tissues of plants of *D. sanguineum* are more or less herbaceous in nature, large-celled and somewhat succulent throughout. The chlorophyl-bearing tissue which, in striking contrast with the condition in *Nicotiana* and *Lycopersicum* (figs. 1 and 2), is continuous over the separation zone, is composed of two rows of small, spherical cells just beneath the epidermis. Except for a layer of collenchyma, whose much elongated cells extend the entire length of the pedicel and thus continue the collenchyma through the separation layer, the cortex and pith are composed of more or less isodiametric, thin-walled cells. Floral abscission is as common in this species as it is in *Nicotiana*. The flowers are very large and furnish excellent material for a study of the cytology of abscission. Unfortunately not a sufficient number of flowers could be obtained to make possible any detailed study of this genus. It was noticed, however, that there is no region of small cells at the base of the pedicel within which separation occurs and that the separation cells are identical in size and shape with those on either side among

which separation does not occur. The separation layer here is located about 8 mm. distal to the base of the pedicel, with absolutely no external indication of its position. Microchemical tests, which in *Nicotiana* gave different reactions in the case of the separation zone and in the case of normal cortical cells, here fail to show any corresponding condition of differentiation.

Abscission has never been found to occur in *Petunia* or *Salpiglossis*, so that it is of interest to examine the histological condition of the base of the pedicel in these two species. They are practically identical with regard to the structure of the pedicel, so that the description given below can be taken as applying to both genera. The cortical cells of the pedicel pass into those of the stem without any groove or small-celled region. On the ventral side, however, is the region of small cells in the axis of the pedicel, which is more or less common to all flowers. The tissues of *Petunia* are not so soft and succulent as those of *Datura, Nicotiana*, and *Lycopersicum*. They tend rather to be dry and tough. The cells in the cortex and pith are also not so nearly isodiametric as in *Datura*, but are much elongated in a direction parallel with the long axis of the pedicel.

The condition in the other species mentioned above will be given only a general description. Abscission occurs in all the other species except *Salpichrora* and *Lycium* which, however, do not differ, in respect to the histology of the base of the pedicel, from any of the others. *Solanum tuberosum* resembles *Lycopersicum*. All the other species are similar in regard to the structure of the separation zone. There is in every case a general region of small cells extending across the base of the pedicel where the separation layer occurs.

3. Development of the Separation Zone in *Lycopersicum* and *Nicotiana*

a. LYCOPERSICUM

The development of the separation zone could be followed better in *Lycopersicum* than in *Nicotiana* because in the former the zone is not so close to the main axis of inflorescence. The problem here resolves itself into an effort to determine, by means of longitudinal sections of very young pedicels, how early in the development of the flower the groove and the differentiation in cell size of the separation cells appear. It was found that the development of the separation zone indicates the method by which the groove and differentiation in cell

size originate. The groove is fairly well developed (fig. 5) in young buds whose corolla is only 3 mm. in length, but is not so deep as in older buds. The cells of the separation zone at this stage are smaller than cells on either side, but the difference is not so prominent as in older flowers. In very small buds whose corolla is only 1 mm. in length or whose calyx is only 2 mm. long, the groove is just beginning to appear (fig. 4). In buds below this size (fig. 3) no groove or differentiation in cell size can be detected. Abscission can occur in these early stages, before the groove or differentiation in the size of the separation cells has appeared, as well as at any other stage. In these early stages the radial diameter of the cortex is much less, as compared with that of the pith, than in older flowers. It is evident, therefore, that the cells of the separation zone are small because they retain their original small size while the rest of the cortical cells increase in size. The fact that the groove is formed makes it probable that there have been few cell division, or none, in the separation zone of the cortex during the development of the bud. It was observed, however, that the cells of the separation zone in the pith retain their meristematic nature for a considerable period during the development

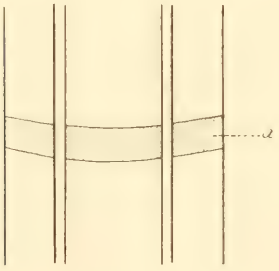

Fig. 3

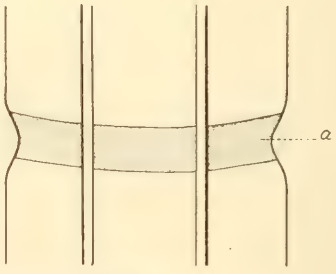

Fig. 4

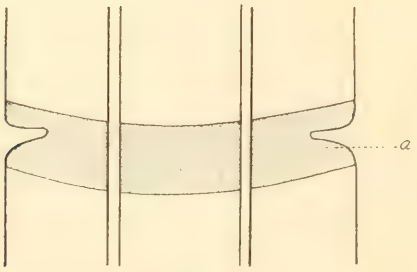

Fig. 5

a—separation zone

of the flower. They are at this time rectangular in shape, elongated perpendicularly to the long diameter of the pedicel, and arranged in longitudinal rows. In later stages, however, when the flower is at anthesis, or the fruit is forming, these cells have rounded up and become irregularly arranged, thus leaving rather large intercellular spaces.

b. NICOTIANA

The separation zone develops in *Nicotiana* much as it does in *Lycopersicum*. It was observed in very young buds—calyx 2 or 3 mm. or shorter—that no groove was present. In buds larger than these, the groove and small size of the separation cell is apparent, appearing first on the dorsal side of the pedicel. It is evident that in both these genera the groove and area of small cells are explained in the same way, i.e., by the fact that the normal cortical cells increase in size faster than do the cells of the separation zone. Since in both genera abscission can occur even before differentiation of any kind appears at the base of the pedicel, it is evident that the groove and small-celled region do not necessarily bear any relation to abscission. This statement is borne out by the fact that in *Datura* neither the groove nor the area of small cells is present and in *Nicotiana* separation occurs a short distance distal to the groove.

c. CONCLUSIONS FROM THE STUDY OF THE DEVELOPMENT OF THE SEPARATION ZONE

In view of the above discussion it is clear that the separation layer in *Lycopersicum, Nicotiana, Datura,* and probably in the other genera noted, originates according to the first method, *a*, proposed by Kubart (cf. page 350). That is to say, the separation layer represents merely a portion of the primary meristem which retains its original physiological capacities.

4. INCREASE IN SIZE AND DEVELOPMENT OF MECHANICAL TISSUES IN THE PEDICEL OF *Nicotiana* AND *Lycopersicum*

There is a marked increase in the size of the pedicel in both *Nicotiana* and *Lycopersicum* during the development of the fruit. It was found that during this development the diameter of the pith remains about the same, the actual increase in size being almost entirely confined to the cortex (cf. figs. 3, 4, and 5). This increase in the diameter of the cortex in the pedicel of *Nicotiana* is due, in the first place, to an increase in the size of the original cortical cells,

which in average cases measured about 20μ in diameter in the flower and about 40μ in the fruit. In the second place, it is due to four or five divisions of the cambium layer. This second factor in the increase in size of the pedicel becomes evident when a count is made of the cells between the phloem and tracheæ, the result giving approximately six cells in the flower and eleven in the fruit.

The increase in size of the pedicel of *Lycopersicum,* which is much more prominent than the increase in *Nicotiana*, can be explained in the same manner. In the former the increase in size, which in this case takes place almost entirely distal to the groove, may proceed to such an extent that the diameter of the pedicel of the fruit is two or three times that of the flower at anthesis. A measurement of the cortical cells in cross-section gave on the average 10μ in the flower and 28μ in the fruit. In this case only two or three divisions of the cambium occur; the cells resulting immediately show lignification.

The next subject of consideration is the development of mechanical tissue in the pedicel of *Nicotiana* and its relation to abscission. It will be remembered that there was no mechanical tissue noted in the pedicels of buds and flowers. Parallel with the development of the fruit, however, a continuous ring of mechanical tissue appears in the xylem of the pedicel. This mechanical tissue is evidently the result of a gradual lignification of the cells of the cambium and the outside portion of the xylem parenchyma. There is thus formed a continuous sheath of what may best be called wood-fibre tissue, in the form of a cylinder just outside the tracheal elements. These mechanical elements first appear in the tissues of the pedicel five or six days after anthesis, but since the lignification in these more distal tissues is merely the result of the spreading upwards of the lignification in the older parts of the plant, this period depends somewhat on the position of the flower on the inflorescence. It was noticed in *Nicotiana* that the wood-fibre tissue develops on both sides of the separation zone before appearing in the latter, but in time it becomes continuous through the separation layer. By a lignification of the cells between the two points of the crescent of wood in the separation zone, there is also a slight tendency to close this crescent on the ventral side.

Since abscission has not been observed to occur in lignified cells, the question at once arises whether the tough sheath of lignified cells which continues through the separation layer could hold the fruit on the stem even after actual abscission had occurred. Upon looking over any large number of plants in the field it will at once be evident

that such a condition of affairs very often exists. It will be found in many cases, especially on older plants, that although abscission has occurred in the cortex, as evidenced by the presence af a white, powdery substance at the base of the pedicel, the capsule is yet firm on the stem. Indeed, in certain hybrid tobaccos it is common to find most of the capsules in this abscissed condition. The fruit is supported in these cases by the tough mechanical elements of the wood, which also prevent the breaking of the tracheæ and protect the intraxylary phloem. In the pith the tissues may be in a somewhat abscissed condition, but since there is no way for these cells to escape through the sheath of wood they remain for some time in position before finally collapsing.

The development of mechanical tissues takes place in *Lycopersicum* in much the same manner as in *Nicotiana* but with the distinct difference that in the former the wood-fibre tissue does not become continuous through the separation layer. That is to say, in the tomato a break is left in the mechanical tissue in a plane with the bottom of the groove. It is evident here that abscission would cause fall of the fruit in any stage of its development, although in this case it happens that abscission very rarely occurs after two or three days past anthesis. A condition resembling this one in the tomato was observed in other berry-forming species of the Solanaceae such as *Cestrum fasciculatum* and *Solanum verbascifolium,* which often drop their immature fruits by abscission. Abscission, however, very seldom occurs in mature berries of these species, the fruit generally falling away from the receptacle above the calyx.

THE PROCESS OF ABSCISSION

1. General Description of the Process in Several Genera

a. NICOTIANA

The process of abscission in all the species of *Nicotiana* investigated conforms to the usual type involving separation and isolation of cells. Further details of the process were briefly discussed in a preliminary paper (Goodspeed and Kendall, 1916) for certain F_1 species hybrids of *Nicotiana.* It was there noted that cell separation starts in the dorsal side of the pedicel, in the cortex a short distance distal to the groove (pl. 49, fig. 1) and spreads from this point around to the ventral side. The first external indication seems to be a bulging of

the epidermis (pl. 49, fig. 2) over the tissue in which the process is taking place. Simultaneously with the start of abscission in the cortex, the process apparently originates independently in the pith (pl. 50, fig. 1). It was further noted that the number of cells concerned in the process, as a general rule, is greater in the hybrids than in their parents and also that this is true of "automatic" as compared with "spontaneous" abscission. Just beneath the epidermis the cells involved in separation were reported as being from five to ten tiers thick, but as the process approached the vascular tissue the separation layer was evidently reduced in thickness to not over one or two tiers of cells (pl. 52, fig. 1). In the pith a more or less spherical mass of cells is involved (pl. 50, fig. 1). When the separation is completed the flower may remain in position for some time, until the epidermis and tracheal elements are broken by some mechanical agency.

The exposed separation surface of the pedicel was stated to be convex in outline and slightly notched at the tip. Upon closer examination the surface itself was seen to be composed of the protruding, rounded ends of cells with here and there completely isolated cells and broken ends of spiral tracheæ. These isolated cells are apparently normal and do not markedly differ in form, size, or in the nature of their cell inclusions from the same cells before separation. The exposed surface of the attached portion of the pedicel is similar in appearance to that of the detached portion, but is more or less flat in outline. After separation the cells on this surface collapse and probably act as a protective layer.

Following the observations recorded above, which had to do largely with flower-fall in the F_1 species hybrids, a number of species have been investigated in an effort to determine whether or not their mode of abscission differs from that already described.

It may be noted at the start that no marked exceptions were found to the previously described condition, although at least two stages in the process of abscission have been found to be subject to considerable variation. The first of these stages has to do with the place of origin of the abscission process itself. An independent origin in the pith has been demonstrated to occur in a large number of species and occasionally it was found that the first evidences of abscission could be detected here before any similar evidences appeared in the cortex. Again, it was found in most species that cell separation starts first in the ventral cortex although other places of origin were found in several cases. Thus, in *Nicotiana Tabacum* "Maryland" and F_1 H36,

for example, the process originates on the ventral side and may even spread through the large area of storage cells in the axil of the pedicel before reaching the dorsal side. The distance distal from the bottom of the groove at which separation appears is also subject to variation. This variation, however, is not typical of certain species, since it may occur at different times in the same species, evidently as a result of an abnormal stimulation to abscission.

The second part of the process subject to variation has to do with the amount of tissue that may be concerned in actual cell separation. Abscission first becomes complete in a narrow plane between two or three tiers of cells across the pedicel and the flower can be easily shaken off at that time. If, however, the flower remains on the stem, and is kept turgid by the water rising in the unbroken tracheæ, cell separation spreads more and more widely through the tissues of the pedicel, especially in the pith and cortex. It is the extent to which this spreading normally proceeds that varies in the different species. When the process has spread to a considerable extent, a white ring formed by the isolated masses of cells can be seen with the naked eye at the base of the pedicel and a casual inspection indicates that the amount of this white substance varies in the different species. In most hybrids, except F_1 H179, there is more spreading in normal abscission than in pure species. In *Nicotiana quadrivalvis, N. Bigelovii,* and other similar species in which abscission very seldom occurs, no spreading takes place. Spreading, however, occurs to a remarkable extent in *N. Tabacum* "Maryland."

b. LYCOPERSICUM

We may say that, in general, abscission in *Lycopersicum* corresponds to that in *Nicotiana* and that the main points of distinction between the two arise only from the original differences in the separation zones (cf. page 364). In addition, attention must be called to the fact that quite frequently, in individual plants of the tomato, no true abscission occurs in normal flower-fall. In these cases the flower seems to be detached from the plant by a process which compares closely with that called exfoliation. There is no active cell separation and the flower simply wilts and dries back to the groove, where it hangs until broken off by some mechanical agency. The first indication of the process is the loss of chlorophyl in the pedicel, which gradually turns yellow, commencing at the tip and spreading proximal to the separation zone. It is possible that most of the flower-fall

noticed by agriculturists is of this type. Quite often, however, true abscission and this second type of flower-fall may both be found operative in the same plant or even in the same flower. "Spontaneous" flower-fall in the tomato is, of course, of the true abscission type.

Corresponding with the condition in *Nicotiana,* true abscission in *Lycopersicum* is seen to originate frequently in the pith. At any rate, the process goes on here independently of that in the cortex, since the final break is through the tracheæ and epidermis. Furthermore, separation takes place in a plane with the bottom of the groove (pl. 53, fig. 2) whereas, in *Nicotiana,* it takes place a short distance distal to the groove. Separation may at first take place between only two tiers of cells (pl. 53, fig. 2), but in time the process may spread until three or four tiers become involved in separation. However, there is no spreading of the process to a large number of cells, as is frequently seen in *Nicotiana,* so that one very seldom finds the white powdery substance at the point of separation. Also in contrast with the condition in *Nicotiana,* there is, as abscission progresses, no bulging of the epidermis which instead soon breaks in the bottom of the groove. Separation in the tomato takes place in such a way as to give the exposed separation surfaces the same general shape after abscission as in *Nicotiana,* that of the detached portion of the pedicel being convex and that of the remaining portion slightly concave.

c. DATURA

Conditions in *Datura* differ strikingly from those in the two species described above. This would be expected when one considers the great differences in the structure of the separation zones (cf. page 365). In *Datura* there is the usual chlorophyl-bearing tissue, which consists of two rows of small, perfectly isodiametric cells with large intercellular spaces, just beneath the epidermis. It will be remembered from the description on page 365 that this tissue in *Datura* continues the entire length of the pedicel and therefore, in contrast with the condition in *Nicotiana* and *Lycopersicum,* extends through the separation zone. The first sign of abscission is the maceration of this tissue as indicated by the appearance of a white color under the epidermis. The latter may as a result become detached from the tissues of the cortex for a distance of 2 cm. or more along the base of the pedicel. This is soon followed by a break over the separation layer and a curling back of the epidermis on either side, with most of the chlorophyl-bearing cortical tissues still attached to its inner surface.

After the break in the epidermis separation continues in the layer of collenchyma just beneath. The cells of the collenchyma layer, which are much elongated parallel to the long axis of the pedicel (five to eight times as long as wide), separate for a distance of about 0.3 mm. up and down the pedicel, involving only a few tiers of cells. It is evident that the cells of this tissue separate without difficulty, although not by any means as freely as the small spherical cells described above. The large, isodiametric, parenchyma cells of the cortex separate for a distance of 2 or 3 mm., involving many tiers of cells. The cells of the starch sheath, which are small and spherical, separate for a distance of 1 cm. or more, thus causing a longitudinal cavity to be formed just outside of the vascular bundles. In the latter, separation involves only two or three tiers of cells. Separation originates and continues in the pith independent of the process in the cortex, but involves about the same number of cells as in the parenchyma of the latter tissue. When separation has thus become complete, the weight of the flower is very often sufficient to break the tracheæ and cause the flower to fall to the ground.

Several important facts are brought out by this examination of abscission in *Datura*. In the first place, it shows that floral abscission can take place without any structure which might possibly be interpreted as a morphologically differentiated separation layer. In the second place, it indicates that cell separation is possible in several different types of living cells. It also shows that separation takes place more readily in small cells than in large ones and more readily in isodiametric cells than in elongated ones. The theory that the separation layer is not a morphologically differentiated structure, but represents a physiological condition (Lloyd and Loewi), could certainly be well applied in this case.

d. OTHER GENERA

The process of abscission in the other species listed on page 365 is essentially the same throughout. No indications were noted of cell divisions or elongations accompanying abscission. Separation is brought about by means of a separation of small and active cells located in the general region at the base of the pedicel. In all these forms the separation surface of the pedicel is convex in outline, so that the separation layer must lie in more or less of a crescent in the stem at the base of the pedicel. The main difference between these forms and the three that have been described in detail above is found

in the fact that in the former, with the exception of *Solanum tuberosum,* separation occurs in the stem at the very base of the pedicel, whereas in the latter three it occurs through the pedicel a varying distance from the base.

2. Method of Cell Separation

a. General Remarks

It will be remembered that two theories have been proposed to account for the cell separation that is responsible for abscission. First, it is conceivable that cell separation may be caused by an increase in cell turgor, which causes the cells to round up and pull apart without any change taking place in the chemical nature of the middle lamella. Second, cell separation may be caused by a chemical dissolution of the middle lamella with or without an increase in cell turgor. The main difference between the two theories is that the second, in contrast with the first, maintains that chemical alteration of the middle lamella is always necessary before abscission can occur. The first theory gains support from the work of Fitting and the second from the work of Hannig, Lee, Strasburger, and Lloyd. Wiesner, Kubart, and Loewi believe that cell separation takes place by the action of both factors but that either factor may at times be the more important.

b. Cytological Changes Accompanying Abscission

It was stated in a preliminary discussion (Goodspeed and Kendall, 1916) first, that no indication of cell divisions or elongations were observed accompanying abscission, and, second, that no evidence of the dissolution of the middle lamella had at that time been obtained. The first statement has been corroborated in that, during all the later experiments, no divisions or elongations have been observed in any of the described species. The dissolution of the primary cell membrane, however, because of more exact knowledge of the proper time to take sections and of more successful staining methods, has been fairly well established.

The main problem here was to determine by the use of various stains whether or not the primary and secondary cell membranes of the separation cells stain differently in the early stages of abscission than under normal conditions. This was a point which was found very difficult to determine, principally because of the fact that the

separation cells are, comparatively speaking, very small, but also because of the fact that the walls of these cells fail to show any stratification.

Iodine, Delafield's haematoxylin, Ruthenium red, Bismark brown, methylene blue, erythrosin, and eosin were used with little success in most cases. By using iodine, however, just as abscission is known to be commencing, a white streak may be seen across the section in the region of the separation layer. Upon careful examination it was decided that this white streak was due to the failure of most of the cell walls in the separation layer to take the stain. Although it is probable that with more careful examination the other stains mentioned above would give similar results, it was found that methylene blue was the only stain with which anything definite could be established. If a thin longitudinal section cut in paraffin as abscission is known to be starting, and stained in methylene blue, is examined (cf. page 361), it will be found that the walls of those cells in which separation is about to occur have remained almost entirely unstained. The protoplasts in these cases seem to be surrounded only by the thin tertiary membranes, between which is a streak of colorless material of varying width (pl. 51). Cell walls where separation is not expected to occur, however, stain a dark blue throughout in the normal manner.

An examination of freshly isolated cells washed off from the end of an abscissed pedicel shows that these cells are still turgid and active. It was impossible to determine whether these cells had increased in size, as compared with the size of similar cells before abscission, but it is evident that the increase, if any, had not been very great. The cells still contain their large nuclei, and occasional starch grains, and show after isolation no signs of degeneration even after several hours in water. In addition, these isolated cells appear to have retained their original shape. In the collenchyma of *Datura* the cells are from five to eight times as long as wide, and yet these cells retain their original shape when isolated, as a result of the dissolution of the middle lamellae. This isolation has evidently not been complete, since large masses of cells are seen still attached to each other. It is noticed that in all cases the protoplast is surrounded by an extremely thin membranous wall (pl. 52, fig. 3). It is also frequently noticed that the protoplast seems drawn away from the cell wall as if plasmolysis had occurred. It is possible that this appearance may be due simply to the gathering together of granules and the denser portion of the protoplasm in the center of the cell.

c. EXPERIMENTAL EVIDENCE FOR THE DISSOLUTION OF THE MIDDLE LAMELLA

It is supposed that the middle lamella, or primary cell membrane, is largely composed of calcium pectate, a calcium salt of pectic acid which has been given the general name pectose. The secondary cell membranes probably contain a larger proportion of cellulose with the pectose than is present in the primary membranes. This pectose, which is of course insoluble in water, is disorganized by a process of hydrolysis to form pectin. The pectin, which is a colorless mucilaginous substance, is readily soluble in water but is precipitated along with the proteids and enzymes of the protoplast by the addition of alcohol. Thus, if a water extract is made from separation zones during the first stages of abscission, one would expect to get a solution of several substances, among which would be the pectin produced by the dissolution of the pectose in the primary cell membranes. It might be expected that the amount of precipitate obtained from this extract with alcohol would be greater, provided the amount of other substances remained the same, than the amount of precipitate obtained in a similar manner from separation zones in which there had been no abscission and in which no pectin had been formed. Whether or not the increase in the amount of precipitate is due to the added pectin cannot of course be proven without actual chemical analysis, and such an analysis would be difficult because of the very small samples of material obtainable. However this may be, any difference in the amount of precipitate would be of interest.

This experiment and the two which follow are, as far as I have been able to determine, the first of their kind. Apart from this fact, their chief value probably lies in the fact that they suggest a line of investigation which, if carried on in more detail and with better facilities, will undoubtedly lead to important conclusions. These experiments were, however, carried on with as much care as possible and since the results of duplicate tests are in agreement, they give, as far as they go, dependable results.

After several experiments indicating the results given below, the following test experiment was performed:

Experiment 1.—Two water extracts of equal concentration were made from the lots of material. Lot A contained 200 small pieces of the pedicel in which the separation zone was located and in which abscission had started. Lot B contained an equal weight of a similar

number of pedicels in which no abscission had started. The extracts were made up to 10 cc. and the precipitate obtained with 60 cc. of 95 per cent alcohol. The precipitate weighed in the two lots:

A	996 mg.
B	903 mg.

One of the preliminary experiments performed with a weaker alcohol gave results which may or may not be of considerable importance. In this experiment a light, almost invisible precipitate formed in A and no precipitate in B. Whether or not the pectins precipitate in lower percentages of alcohol more readily than the other substances I have been unable to determine. At any rate, the precipitate in this case felt slimy and mucilaginous to the touch and might well have been the precipitated pectin approximately pure.

d. EVIDENCE FOR INCREASE IN TURGOR

It was stated along with other conclusions in the preliminary paper (Goodspeed and Kendall, 1916) that from the evidence at that time available it was probable that cell separation is caused merely by an increase in cell turgor, and throughout this later work it has been clear that increased turgor is present during abscission. In view of the evidence given above, however, it would seem that turgor can play only a secondary rôle, although the occurrence of increase in turgor must not be ignored.

The bulging of the epidermis frequently noted as accompanying abscission is evidence of increased internal pressure. In the pith the cells next to those which are separating are in a collapsed condition due to the pressure of the expanding separating cells. By various experiments it can be shown that humid conditions favor and severe drought prevents abscission. Richter and others have shown that narcotic vapors which cause abscission also cause increased turgor by increasing the proportion of sugar in starch-containing cells. This increase in cell turgor becomes so great as to cause complete maceration in certain types of tissues. The frequent presence of starch grains in the separation layer of *Nicotiana*, part of which are probably converted into sugar as a result of subjection to illuminating gas, indicates that there is probably an increase of turgor during abscission, at any rate when induced by illuminating gas.

On the other hand, a more extensive examination of abscission in certain plants indicates that all evidences of increased turgor may at

times be absent. Such cases might be explained by the absence of any considerable amount of starch in the cells concerned. Indeed, the starch grains usually noted in the separation layer can not at times be observed. This might also explain the fact that the bulging of the epidermis and collapse of cells in the pith usually accompanying abscission are sometimes absent. Also, starch grains are rarely observed in the separation cells of *Lycopersicum* and *Datura* and in these forms very little bulging of the epidermis occurs. Although humid conditions favor abscission and drought prevents the process, it has also been observed that drought has to be very severe before it produces such a result. Other evidences for increased turgor derived from the turgid appearance of the cells are mostly obtained after abscission has started and, granting that the cells are isolated by dissolution of the middle lamella, more or less expansion due to release of pressure is to be expected.

A critical examination of the separation cells during abscission brings out several facts, other than those mentioned in the above paragraph, which of themselves render inadmissible the theory that cell separation is brought about by increased turgor. These are as follows: 1. There seems to be no perceptible change in cell shape or size during separation. 2. The increase in size of the intercellular spaces does not necessarily take place first between the walls at the "corners" of the cells, but may appear first as a longitudinal streak between the lateral walls of the cells (pl. 51). 3. Cell isolation may be incomplete in large numbers of cells still remaining attached to each other. 4. Cell separation first becomes complete in a narrow plane between only two tiers of cells before spreading later to a larger number of cells. 5. The spreading of cell separation itself is obviously hard to explain on the basis of the turgor theory.

In view of the facts brought out in this discussion and the positive evidence for the dissolution of the primary membranes, it should be clear that increase in turgor, at least in the Solanaceae, is not the direct cause of cell separation. Undoubtedly there is often great increase in turgor during abscission, especially in certain types of cells, but this increase, instead of being the direct initiating factor, probably serves merely to hasten and facilitate the process.

c. EXPERIMENTS ON THE AMOUNT OF SUGAR IN THE STEM AND PEDICEL OF *NICOTIANA* DURING ABSCISSION

After several experiments, all of which indicated the results obtained below, the following experiment was performed. Experiment 2*a* was devised to show the change in the amount of sugar which occurs in the tissues of the pedicel during abscission. Experiment 2*b* was intended to show this same difference in a restricted region of the stem just proximal to the separation layer.

Experiment 2a. Lot A included 200 pedicels of flowers which had fallen a few minutes before being collected as a result of being subjected to illuminating gas. Lot B included 200 pedicels of flowers picked at the same time as those making up Lot A, but in which no abscission was induced. The water extracts made with 10 cc. from equal weights of the two lots were tested with surplus Fehlings solution. The precipitates formed upon boiling weighed:

A	68 mg.
B	95 mg.

Experiment 2b. This experiment was carried out in the same manner as experiment 2*a*, but the precipitates in this case were of such small quantity that no attempt was made to get actual figures as to their weights. It was clear, however, merely from an examination of the filter paper, that there was more precipitate in B than in A—just the reverse of Experiment 2*a*. The difference was evidently not as great as in the latter experiment.

Experiment 2*a* seems to indicate that during abscission there is a reduction of nearly one-third the normal amount of sugar in the pedicel. Other preliminary experiments performed as abscission was starting showed only a slight reduction in the amount of sugar in the pedicel. Thus possibly the withdrawal of sugar commences with the start of abscission. Experiment 2*b* indicates that there is probably a slight increase in the amount of sugar in the limited region proximal to the separation during abscission. It is possible that most of the withdrawn sugar is used as a source for the energy required in the active process of cell separation. The slight increase proximal to the separation layer also shows that there is probably an increase in cell turgor in the actual tissues which contain the separation layer, due to the conversion of starch into sugar.

f. POSSIBLE AGENCY ACTIVE IN THE DISSOLUTION OF THE MIDDLE LAMELLA

The pectose of the middle lamella may be broken down into the soluble pectin in three different ways—by the action of an acid, of an alkali, or of the enzyme pectosinase. Since it is doubtful whether alkaline reactions in living cells frequently get strong enough to affect the middle lamella, the probable active agency is limited to the acid or the enzyme action. Up to the last few years very little has been known about the action of enzymes concerned in pectic digestion. It has been natural, therefore, for investigators (cf. Wiesner, 1905, and Kubart, 1906) to consider the acid as probably the active agency. In this connection, it is well to state that I have obtained distinct acid reactions with litmus from the base of the corolla of *Nicotiana* during abscission. This would confirm Kubart, who, it will be remembered, obtained similar reactions from the corolla of *Nicotiana*. But in this case I sometimes obtained acid reactions from the corolla when in the normal condition. Since these observations offer no detailed evidence that acidity has increased during abscission to a degree higher than normal, their significance can well be doubted.

The tissues of *Datura* give a distinct acid reaction to litmus in the normal condition. Experiment 3 below shows a slight increase in acidity during abscission. No acid reactions of much intensity are given by the base of the pedicel of *Nicotiana* either in the normal or abscissed condition.

Experiment 3. Lot A contained the bases of three pedicels cut while abscission was going on. Lot B contained an equal weight (6 gm.) of the bases of three pedicels cut in the normal condition. These were extracted with water and the extracts made up to 10 cc. each. By titration with 10 per cent NaOH and phenolphtalein the following results were obtained:

A..... 0.75 cc. required to neutralize
B..... 0.6 cc. required to neutralize

A similar experiment on *Nicotiana* showed, however, that the normally low acidity of this genus is slightly reduced during abscission, as indicated by the following results:

A..... 0.25 cc. required to neutralize
B..... 0.37 cc. required to neutralize

The normal acidity in *Datura* is high, but it is doubtful whether the increase is large enough to account for the dissolution of the middle lamella. At any rate, it is certain that acidity does not enter into the problem in the pedicel of *Nicotiana*. We must, therefore, fall back upon the enzyme action as probably responsible for the process of cell separation.

Most hydrolysing processes characteristic of living cells are now supposed to be due to the action of enzymes of different kinds. It has been definitely claimed (cf. Atkins, 1916) that an enzyme which has been called pectosinase is capable of breaking down the pectose of which the middle lamella is composed. Add to this the fact that the action of enzymes has been shown, as has also the process of abscission, to be very sensitive to all kinds of changes in the external environment, and it is fairly safe to assume that the method of cell separation is fundamentally an enzyme problem. Irrefutable proof of this could be obtained only by testing for the activity of pectosinase during the early stages of abscission and by demonstrating the absence or inactivity of this enzyme in species where abscission does not occur.

ABSCISSION OF THE STYLE AND COROLLA

Abscission of the corolla in *Nicotiana* was described by Kubart and it may be said at once that the observations herein described agree entirely with his. Abscission of the corolla is brought about by the separation, without any previous cell divisions or elongations, of living cells at the base of the corolla tube. The separation layer, which is in no way morphologically differentiated from the neighboring tissue, is located about 1 mm. from the point of insertion of the corolla on the receptacle. It thus occurs in the distal part of a region where intergradation of cell shape, between the isodiametric cells of the receptacle and the more or less elongated cells of the corolla, is apparent. The separation cells which are in this region of intergradation are not isodiametric but are more or less elongated parallel to the long axis of the corolla. All the cells in cross-section of the

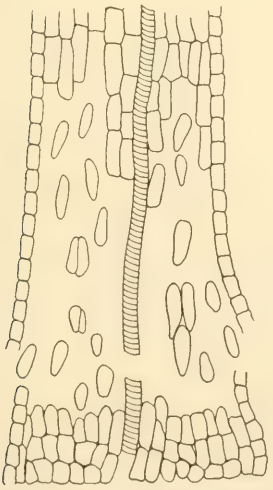

Fig. 6. Longitudinal radial section of the base of the corolla tube of *Nicotiana*, showing the method of abscission.

base of the corolla tube at about the level of the separation layer seem to be involved in the process except the epidermal cells and the tracheæ. The process of cell isolation in this case may spread up and down for quite a distance between the epidermis and tracheæ, thus involving a large number of cells (fig. 6).

Abscission of the corolla in *Datura* differs slightly from that in *Nicotiana.* As in the latter, there is no differentiated separation layer, separation occurring in cells which are not visibly different from other cells of the corolla. Cells more or less elongated are involved, as in *Nicotiana,* but in *Datura* the region of separating cells is limited to certain tissues—that is to say, not all the cells across the base of the corolla tube at about the level of the separation layer are involved in the process of abscission. The base of the corolla in *Datura* is characterized by distinct longitudinal ridges which alternate with deep grooves. Thus, a cross-section of a portion of the base of the corolla appears as in fig. 8. Cell separation fails to occur in the outside ridges at the level of the separation layer, so that, looking at the base of the corolla tube from the outside during abscission, one sees separate crescent-shaped regions of macerating cells alternating with cells which are not separating (fig. 7). This is explained when a cross-section is taken (fig. 8), which shows that several vascular bundles, the cells of which do not separate, are collected in the outside ridges.

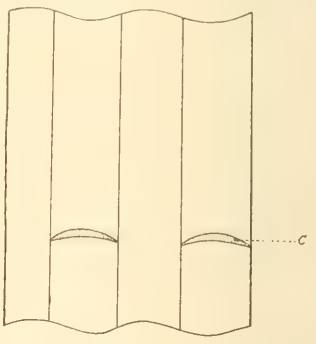

Fig. 7
c—region of separating cells.

Abscission of the style occurs normally in *Nicotiana* and *Datura* a short time before the corolla has fallen. So far as it was possible to determine, the process of abscission is exactly the same in the style as in the corolla. A separation of very small, more or less elongated cells takes place at the base of the style without any external indication such as frequently occurs in the pedicel of the

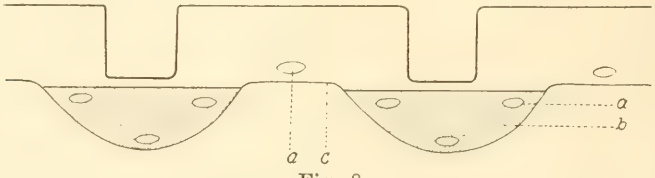

Fig. 8
a—vascular bundle.
c—region of cell separation.
b—region of no cell separation.

flower. As in the case of the corolla, there is no structure which might be interpreted as a differentiated separation layer. Separation occurs in a region of intergradation in cell shape between the spherical cells of the ovary and the cells of the style, which are elongated parallel with the long axis of the style.

TIME OF ABSCISSION

1. Reaction Time

a. REACTION TIME IN NORMAL ABSCISSION

The term "reaction time" is used in referring to two rather distinct subjects. First, we may have a reaction time represented by the time intervening between anthesis and normal abscission due to lack of fertilization. Second, we may have a reaction time which has to do with the period between the application of the stimulus and flower-fall in "spontaneous" abscission. The reaction times in normal abscission were discussed in an earlier communication in the case of two F_1 species hybrids of *Nicotiana* and their parents. The statements there made have been repeatedly verified and in addition a considerable amount of data has been accumulated in regard to the time of abscission in other species of *Nicotiana* and in the genus *Lycopersicum*. In the case of the former the observations were also made upon the abscission of the corolla, the effect of pollination on reaction time, and the reaction time in "spontaneous" abscission.

In determining the abscission times for the hybrid F_1 H154 (cf. page 386), a great variation was noted in the normal reaction time. In the case of the hybrid F_1 H179 (cf. page 386), however, and in other species or varieties investigated, very little variation in the time of abscission has been noted. The range of variation in these species and varieties practically always falls within two or three days and a large number of observations gives identical times, as far as the number of days is concerned, in the case of seven to ten flowers. There is a certain variation in the length of the reaction times in different flowers on the same plant, but the plants of a species do not differ from one another in their average reaction times. It was noticed that the figures were approximately the same whether the averages were based on the records of four or five flowers or of a considerably larger number; thus the results given in the following table may be considered conclusive. Where the number of flowers involved is less than four,

however, the results serve merely as approximate estimate of the abscission reaction time.

In obtaining the records tabulated below a separate tag was supplied for each flower. This tag was put on the flower at the beginning of the observation, the plant visited twice a day thereafter and records kept on the tags, which were left on the flower until the close of the observation. If a flower fell upon being tapped or shaken, abscission was considered to have occurred and the date was recorded on the tag, which was then collected. Similarly, in the case of the records for abscission of the corolla, a slight pull had to be applied before it could be determined whether abscission had occurred. As a means of preventing fertilization, the stigma was cut away in addi-

TABLE 1

Designation of species or variety	I Time from bud[1] to anthesis		II Time from pollination to mature fruit		III Time from pollination to abscission of corolla		IV Time from anthesis to abscission of corolla, unpollinated		V Time from anthesis to normal flower[3] fall	
	No. flowers	Avg. No. days	No. flowers	Avg. No. days	No. flowers	Avg. No. days	No. flowers	Avg. No. days	No. flowers	Avg. No. days
F_1 H38									10	18
F_1 H179	4	8	4	14	15	3	23	6	20	7
							8[2]	6		
F_1 H36					7	4	3	6		
N. sylvestris			10	11	6	4	9	6	9	15
							4[2]	6		
N. Tabacum "Maryland"			3	12	2	4	2	6	3	5
N. Bigelovii var. Wallacei			5	10	5	3	5	5	8	17
N. Bigelovii "Pomo"			5	19	5	2	6	5	6	no fall
N. Bigelovii var. typica									2	16
									3	no fall
N. Bigelovii (hybrid?)			4	21	1	4	2	8		
N. multivalvis			3	18	2	4			2	no fall
N. quadrivalvis			5	20	5	7	5	2	6	no fall
N. suaveolens					8	8	8	8	7	13
N. Sanderae			15	15	5	4	5	6	6	9
N. rustica var. brasilia							3	4	4	6
N. rustica (Winnebago)			7	6	7	3	4	4		
N. rustica var.?			4	15	4	2	5	3	6	5
Lycopersicum esculentum	4	6			4	3	4	8	17	9

[1] The buds recorded here were of such size that the corolla and calyx were of the same length.

[2] Sterile pollen applied to the stigma.

[3] By normal flower-fall is meant fall due to lack of fertilization.

tion to removing the anthers before anthesis. Various experiments had shown that such an operation on the flower does not induce abscission or affect its normal physiological condition to any great extent.

F_1 H154 is *Nicotiana Tabacum* var. *macrophylla* (U. C. B. G. 22/07) × *N. sylvestris* (U. C. B. G. 69/07). F_1 H179 is *N. Tabacum* "Cuba" (U. C. B. G. 200/14) × *N. sylvestris*. F_1 H36 is *N. sylvestris* × *N. Tabacum* var. *angustifolia* (U. C. G. B. 68/07).

The results given in table 1 indicate, in the first place, that the different species differ considerably in all the types of abscission reaction times considered, and, in the second place, that on the average, application of a fertile pollen to the stigma tends to shorten the time between anthesis and abscission of the corolla by two days. The one apparent exception to this statement is *Nicotiana suaveolens*, but in this case the pollinated flowers fell five or six days later than the corolla, indicating that growth of the pollen had not proceeded very far. Records on F_1 H179 and *N. sylvestris* indicate that sterile pollen does not have the same effect on abscission that fertile pollen does. This would seem to show that here the effect of pollination upon the postfloration phenomena is not due, as Fitting (1909) has found in orchids, to mere mechanical or chemical stimulation of the stigma by the pollen. This much being certain, the question still remains whether the results obtained depend upon fertilization or are due to the growth of the pollen tubes down through the style.

According to East (1915), working on self-sterility in *Nicotiana* hybrids, the pollen tubes reach the ovary, in cases of cross-pollination, three or four days after application of pollen to the stigma. Since in all cases recorded above cross-pollination was carried on and since in most cases the corolla was not thrown off until three or four days after application of pollen to the stigma, it is possible that fertilization is the important factor in shortening the time between anthesis and abscission of the corolla. In *N. quadrivalvis,* however, the corolla was thrown off within eighteen hours after pollination, whereas, when pollination is prevented, the corolla may remain on the flower for fifty-seven hours. If East's conclusions are correct, this would seem to indicate that the shortening of the reaction time in abscission of the corolla is due to some stimulation of the style by the pollen tubes and not to fertilization. This conclusion, however, could be doubted even here, because the style of *N. quadrivalvis* is very short, so that the pollen tubes might reach the ovary in a much shorter time than is

required in larger flowers. An attempt was made to get further data on this point by removing the style several hours after application of pollen before the pollen tubes could possibly have reached the ovary. This operation occasionally causes the whole flower to fall, and since in such cases abscission in the pedicel occurs before fall of the corolla, no results in regard to the latter organ are obtained. The possible effect of the operation on the abscission of the corolla was checked by control tests of unpollinated flowers in which the styles had also been removed. This can also be checked by a comparison with the periods of time given in table 1, column III.

It was found in three flowers of F_1 H179 that, when the style was removed three days after pollination, the corolla was, on the average, thrown off three days after anthesis. The control test for this experiment gave in three flowers an average of five days. Where the style was removed two days after anthesis, four flowers gave an average of three days. Where the style was removed one day after pollination, the corolla was abscissed in five flowers an average of three days after anthesis. A control test gave in this case an average of five days for five flowers. Finally, the style was removed in seven flowers seventeen hours after pollination. The seven flowers gave in this case an average of four days for the time between anthesis and fall of the corolla. A control test for this last case gave for five flowers an average of five days.

These experiments were repeated with *N. sylvestris*. In one case where the style was removed in three flowers two days after pollination, the corolla was thrown off on an average of four days after anthesis. A control test of this case gave an average of six days for three flowers. In another case the style was removed in three flowers one day after pollination. In this case the corolla was abscissed on an average of three days after anthesis.

The results given in the above paragraphs indicate definitely that it is the stimulation of stylar tissues caused by the growth of the pollen tubes which shortens the time between anthesis and abscission of the corolla. They also show that the removal of the style has no appreciable effect on the abscission of the corolla. It is evident from the results given that the influence of the pollen is seen as early as seventeen hours after pollination, and it is possible that the effect may be manifested even earlier. It is significant that the period given in the case where the style was removed seventeen hours after pollination is one day longer than in the case where the style was removed

twenty-four hours after pollination. This may possibly indicate that in the first case the influence of the pollen tubes has diminished, because of the shortening of the period which they have had for growth. If this is the case, it is reasonable to suppose that the influence of the growing pollen tube increases up to twenty-four hours after pollination as the pollen tube lengthens. Thus, at six hours after pollination it is possible that no effect of the pollen tubes would be noticeable, while twenty-four hours after pollination the entire influence of the growing pollen tube has been exerted.

The effect of pollination on the time between anthesis and flower-fall was tested by experiments similar to those described above. Results in such experiments are difficult to obtain because removal of the style frequently causes the premature fall of the flower. If the flower fell before abscission of the corolla, the fall was considered premature, as the result of the removal of the style, and the record of that particular flower not considered. Since under ordinary conditions pollinated flowers remain on the plant, it is to be expected that the stimulation of the stylar tissues by the pollen tubes, if it has any influence at all, would increase the length of time between anthesis and flower-fall. Granting the truth of this assumption, any reduction in time between anthesis and fall can be considered as the result of removal of the style.

In one test on ten flowers of F_1 H179, where the style was removed two days after pollination, flower-fall occurred on an average of seven days after anthesis. A control test in this case also gave seven days for ten flowers. This time is approximately the same (the actual average calculated to the tenth of a day was 6.7) as those given in table 1, column V, for the time between anthesis and normal flower-fall due to lack of fertilization. A similar test on six flowers of *N. sylvestris,* where the style was removed two days after pollination, gave an average of thirteen days. The time for this species in table 1, column V, is fifteen days.

These two records indicate that the stimulation of the stylar tissues by the growing pollen tubes has no effect on the time between anthesis and flower-fall. In the second case above, and also perhaps in the first, the stimulation of the style seems to have shortened the time somewhat, but in this case the result can be explained by the effect of the later removal of the style.

b. REACTION TIME IN "SPONTANEOUS" ABSCISSION

Exact data in regard to the reaction time can be given only in two definite cases. The observations in these cases were made on small shoots of the plant to be considered, which were placed in water and inserted under a bell-jar containing 1.5 per cent illuminating gas. After several hours, the material was shaken every fifteen minutes to determine when the first flower fell. F_1 H179 and *N. Tabacum* "Maryland" were selected as material for the experiments because these forms were found most sensitive and thus react regularly and quickly to stimuli. Abscission occurs in the pedicel of F_1 H179 seven hours after insertion into 1.5 per cent illuminating gas at a temperature of approximately 19° C. The smaller buds begin to fall first, but are followed in a short time by the open flowers. Abscission occurs in *N. Tabacum* "Maryland" in eight hours under the above conditions.

The remainder of the data having to do with the reaction time in spontaneous abscission is in the form of approximate estimates derived from the results of experiments on the induction of abscission. In the case of abscission induced by illuminating gas most species which shed their flowers in 1.5 per cent illuminating gas do so after ten or fifteen hours at room temperature.

There remains now to be considered the reaction time in cases of flower-fall due to mechanical injury. The results along this line are largely derived from tables 2, 3, 4, and 5, which, however, were arranged to show more particularly the comparative effect of different types of injury, as causing or not causing abscission in flowers of various ages. These tables might as well be presented under the heading "Experimental Induction of Abscission by Mechanical Injury" (page 405), but since it is necessary to draw certain conclusions from them in regard to the time of abscission they are presented and explained at this time.

Tables 2, 3, 4, and 5, which follow, serve to record the results of a number and variety of experiments all designed to show the relation of mechanical injury to abscission. It was very soon discovered while carrying on the experiments that the effect of injury depends to a large extent upon the age of the flower. Now the age of the flower can be most conveniently measured by determining the increase in size of growing parts such as the corolla and ovary. Thus it was necessary in each case to record the size of the flower—size being a

criterion of age—upon which the test was being made. This was done by noting on the tag which was supplied for each flower (cf. page 385) the length of the corolla in millimeters, the condition of the corolla, or any other condition of the flower which would serve to indicate its age. The period of development of the flower and fruit is divided into several arbitrary stages, each of which is designated by a Roman numeral in the second column of the tables. Where the number of flowers designated in the first column are nearly in the same stage of development only one numeral appears in the table, but where the range in size of the flowers is quite extensive two numerals appear, representing the range in size within which the flowers were found at the time of the experiment. The stages of floral development which each Roman numeral represents are given below.

Bud

I	corolla 2 mm. to 5 mm. in length
II	corolla 6 mm. to 10 mm. in length
III	corolla 11 mm. to 15 mm. in length
IV	corolla 16 mm. to 20 mm. in length
V	corolla 21 mm. to 30 mm. in length
VI	corolla 31 mm. to 40 mm. in length
VII	corolla 41 mm. to 50 mm. in length

Flower

VIII	corolla opening
IX	anthesis
X	2 days after anthesis
XI	corolla withering

Fruit

Immature

XII	fruit 5 mm. to 8 mm. in length
XIII	fruit 9 mm. to 10 mm. in length

Mature

XIV	fruit 11 mm. to 12 mm. in length

The operation of injuring the flower consisted largely in removing, by cutting away with a sharp safety razor blade, entire floral organs or parts of them. In some cases, however, organs were only slit longitudinally with a sharp knife or merely punctured with the point of a pair of forceps.

Several types of injury that remove the style, stigma or stamens before pollination may cause fall by preventing fertilization. It is evident, therefore, that fall occurring after such an operation performed on the flower before anthesis may be due to lack of fertilization and not to the injury. If, however, the fall occurs within the minimum time elapsing between anthesis and normal flower-fall due

to lack of fertilization, it can be safely concluded that the fall is due to the effect of the injury. This minimum time is about seven days for *N. Langsdorffii.* It can be safely said, therefore, that any fall occurring in less than seven days after injury to the flower near anthesis is due directly to the effect of the injury. In cases where the stamens or style are removed in flowers younger than those at anthesis,

TABLE 2

Effect of Different Types of Injury in Causing Flower fall in *N. Langsdorffii* var. *grandiflora*

	No. flowers	Size or condition of flowers	Injury to					Avg. No. days before fall of remaining organs
			Calyx	Corolla	Stamens	Pistil	Pedicel	
a	10	II-VII	all cut	all cut	all cut	all cut		1
	10	VII-XI	"	"	"	"		2
	10	XII-XIII	"	"	"	"		3
	2	XIV	"	"	"	"		no fall
b	4	I-II	½ cut	⅔ cut	"	style cut		7
	3	III-VII	"	"	"	"		7
	3	VIII-IX	"	"	"	"		7
	5	XI-XII	"	"	"	"		no fall
	2	XI-XII	"	"	"	style and part of ovary cut		5
	3	XIII-XIV	"	"	"	"		4
c	5	V-VII		½ cut	"	⅔ style cut		7
	5	VIII-IX		"	"	"		6
	2	XI		"	"	"		9
	3	XII		"	"	"		no fall
d	4	I	all cut					3
	1	II	"					no fall
	12	III-XII	"					"
e	2	V-VII	slit on sides to base	2 slit on 2 sides to base				7
	8	III-XI	"	"				no fall
	3	II	"	"		ovary slit		3
	3	V-VII	"	"		"		5
	3	IX	"	"		"		2
	2	XII	"	"		"		5
f	5	IX			anthers all cut			10
	2	V-VII			all cut			7
	3	VII-X			"			9
g	4	VIII				stigma cut		9
	2	V-VII				style cut		7
	2	VII-X				"		10
h	4	XIV	all cut			all cut		no fall
	2	XIII	½ cut			½ cut		2
	2	XIV	"			"		no fall
	4	XIII-XIV	slit to base			slit to base		"
i	10	II					slit to base	"
	3	IX					"	"
	6	I-VIII					1 cut ½ through	"
	8	II-XII					2 cuts ½ through	"

allowance must be made for the approximate number of days preceding anthesis. Thus, if a flower of the above species is injured three days before anthesis, the fall can not be assigned to the injury unless it occurs before ten days have elapsed. The minimum time for F_1 H179 is about five days; thus, any time of five days or more recorded on a flower, injured near anthesis, was considered as "no fall." The minimum time for *Lycopersicum* is about six days.

Finally, it is necessary to state that the process of reaction to the different types of injury recorded in the following tables was by no means impeded by low temperatures. *Nicotiana Langsdorffii* was tested out in a greenhouse where the average temperature approximated 75° F. The tests on F_1 H179 and *Lycopersicum* were performed in the botanical garden of the University during July and August, when the temperature was also comparatively high.

The following statement of results is derived in great part but not entirely from the foregoing tables. It has been noticed that cutting off the freshly opened flower at the tip of the pedicel causes the remainder of the pedicel to be thrown off in from ten to fifteen hours, but after the same operation on developed capsules the pedicel remains firm from thirty-six to ninety-six hours after the injury. Removal of the calyx causes the fall of buds in two or three days, depending upon the age of the bud. Removal of half the calyx together with two-thirds of the corolla and all the stamens causes fall in one to four days, depending upon the age of the flower. A

TABLE 3

EFFECT OF POLLINATION OF FLOWERS OF *N. Langsdorffii* var. *grandiflora* ON REACTION TO INJURY

	No. flowers	Pollination	Injury	Avg. No. days before fall
a	2	pollinated when injured	calyx and stamens cut	no fall
	2	not pollinated	"	10
b	4	pollinated when injured	calyx " ½ corolla cut	no fall
	5	not pollinated	"	8
		No. days after pollination when injured		
c	2	1	all organs cut at tip of pedicel	2
	2	2–6	"	2
	2	7–8	"	2
	3	2	½ calyx, ⅔ corolla, stamens, style cut	4
	3	4–5	"	5
d	2	6–7	"	2
	1	9	"	3
	1	9	"	no fall

transverse cut through the entire flower which passes through the middle of the ovary causes fall in one to two days. A similar operation in the case of maturing fruits changes the date of fall to four to eight days. Removal of half the corolla and all the stamens causes fall of buds in one day and the fall of young flowers in two to three days. Removal of the stamens or style in buds causes fall in

TABLE 4

Effect of Different Types of Injury in Causing Flower fall in F_1H179

	No. flowers	Size or condition of flowers	Injury to					Avg. No. days before fall of remaining organs
			Calyx	Corolla	Stamens	Pistil	Pedicel	
a	9	II-VIII	½ cut	⅔ cut	all cut	style cut		1
	6	XI-XV	"	"	"	"		no fall
b	4	III-VII		½ cut	"			1
	10	VIII-IX		"	"			no fall
c	10	V-VIII		"				"
d	9	I	all cut					2
	7	II	"					2
	3	II	"					no fall
	4	III-IV	"					3
	6	III-IV	"					no fall
	1	V	"					2
	4	V-VII	"					no fall
	2	IX	"					"
e	7	III-IV			"			2
	1	V			"			5
	3	V			"			no fall
	6	VI-VII			"			"
f	5	II-VIII				"		2
	4	VII-VIII				"		no fall
g	1	II	1 slit on 2 sides to base	1 slit on 2 sides to base				5
	1	II	"	"				no fall
	9	IV-VII	"	"				"
	9	II	2 slits on 2 sides to base	2 slits on 2 sides to base				1
	2	II-IV	"	"				4
	5	V-VII	"	"				no fall
h	5	II-V	punctured on both sides	punctured on both sides		ovary punctured, small hole		2
	3	VI-VII	"	"		"		no fall
	3	VII-XI	"	"		"		2
	3	II-III	"	"		"		2
	3	VI-X	"	"		"		2
i	15	III-XII					1 slit to base	no fall
	5						punctur'd many times	1
j	6	XIV	½ cut			capsule ½ cut		4
	3	XIV	"			"		no fall

two to four days. Severe injury of any kind to the ovary causes fall in one to two days.

The figures given above for the reaction time in cases of abscission following mechanical injury, together with a more detailed consideration of the tables, indicate that the reaction time, in general, does not depend so much on the type of injury as on the age of the flower concerned. What connection there is between the type of injury and the reaction time seems to be based, except in cases of injury to the ovary, on the relation of the amount of material removed to the amount remaining. Thus, cutting off the flower at the tip of the pedicel causes abscission of the remaining pedicel more quickly than any other type of injury. One exception to this statement is seen, as

TABLE 5

EFFECT OF DIFFERENT TYPES OF INJURY IN CAUSING FLOWER FALL IN *Lycopersicum esculentum*

	No. flowers	Size or condition of flowers	Injury to					Avg. No. days before fall of remaining organs
			Calyx	Corolla	Stamens	Pistil	Pedicel	
a	4	I	all cut					no fall
	4	II-VIII	"					"
	6	XII	"					"
b	3	XII				entire ovary cut		2
	3	XIII-XIV				ovary punctured 4 times on top		no fall
	1	XII				"		3
	4	XII				ovary punctured 4 times on side		2
	3	XIV				"		no fall
c	4	II	punctured at base	punctured		ovary punctured once on side		9
	4	VIII	"	"		"		4
d	4	II-VIII	½ cut	½ cut				no fall
	4	VIII-IX	"	"		"		5
	3	I-II		"		ovary ½ cut		1
	3	VIII	"	"		"		3
	2	IX	"	"		"		2
e	5	I-IX	"		all cut			no fall
f	5	VIII				style cut		5
	6	X-XI				"		no fall
g	5	VIII-XIV					slit	"
h	3	VIII		all cut	"			4
	5			"	"			no fall
	4	II-VIII		"				"

indicated above, in the case of injury to the ovary in which this organ may be merely punctured, without necessarily removing any material, yet abscission occurs in one to two days after the injury.

It has, on the other hand, been evident throughout all the abscission experiments that age of flower is the important factor in determining the reaction time, older flowers nearly always responding more slowly to stimulation by injury than younger ones. It will be seen, however, from the tables that there are occasionally individual exceptions to the general rule. These exceptions might be explained in a number of ways. For example, it is possible in the case of older flowers that the ovary, having increased in size, was accidentally cut in the operation of injury, thus adding the extra factor of stimulation of the ovary which in younger flowers would not be present. In general, such exceptions to the general rule indicate to what extent the normal or abnormal physiological conditions of the plant enter into the problem.

2. Abscission Time

The abscission time, or the actual time involved in the process of cell separation, was considered in a preliminary paper (Goodspeed and Kendall, 1916) wherein the minimum time in which abscission was known to have occurred was stated to be from four to eight hours in normal abscission and from one to four hours in "spontaneous" abscission. A few additional data are now at hand in the case of F_1 H179 and *Nicotiana Tabacum* "Maryland." These two forms, as has already been noted, are a little more sensitive than most *Nicotiana* varieties and normal abscission was found to take place in from three to six hours.

The time of cell separation in "spontaneous" abscission can be more exactly determined than that in normal abscission because of the regularity with which the plants respond to certain conditions of injury or to the presence of narcotic vapors. Data on this point were obtained in the following manner. Flowering shoots with flowers of different sizes were cut, placed in water and inserted under a bell-jar. Enough illuminating gas was then introduced under the jar to make 1.5 per cent approximately. The temperature during the experiment was practically constant at 19° C. After the shoot had been left in this abnormal atmosphere for five hours a few flowers were picked off at fifteen-minute intervals and free-hand sections made of their pedicels until flowers about the size of those which were being sec-

tioned began to fall. It was found that signs of abscission hardly ever appeared until thirty to forty minutes before actual fall occurred. This indicates that the actual process of cell separation in F_1 H179 takes place in from thirty to forty minutes. Experiments carried on in the same manner with *N. Tabacum* "Maryland" indicate that abscission here takes place in from forty-five to sixty minutes.

Both the reaction time of abscission and the actual abscission time are profoundly influenced by temperature and by humidity. Variation in the intensity of the illumination, however, seems to have no direct influence upon abscission. In comparing the effect of changes in temperature and humidity it was found that the results of experiments intended to show the time of abscission are far more dependent upon temperature than upon humidity. This is not because changes in humidity have little influence upon abscission but because such changes have to be very great indeed before bringing about any appreciable effect. Very slight changes in temperature, on the other hand, often influence abscission to a marked degree. Abscission goes on very actively under high temperatures and conversely very slowly under low temperatures. It starts in the case of F_1 H179 about seven hours after insertion in 1.5 per cent illuminating gas at a temperature of 19° C. If the same experiment be repeated in a temperature of approximately 9° C. abscission may not occur for fifteen to twenty-four hours.

Drought has to be quite severe before retarding abscission. There is no doubt, however, that wilted shoots will not drop flowers as quickly as fresh ones and if the wilting proceeds far enough no abscission will occur. This effect is all the more noticeable if the air around the wilted shoot is kept free from moisture.

EXPERIMENTAL INDUCTION OF ABSCISSION

1. Induction by Illuminating Gas

The first subject to be considered under this heading is the comparative effect of illuminating gas in causing abscission in several species of the Solanaceae. The method of determining this consisted largely in placing flowering shoots of the different species in water under bell-jars and introducing enough illuminating gas under the jars to make the percentage of narcotic vapors in the air around the plant 1.5. The temperature during the experiments was compara-

tively high, ranging from 15° to 20° C. The results, which were recorded approximately fifteen hours after subjection to the gas, are given in the following table:

TABLE 6

Species, variety, or hybrid	Amount of abscission, expressed almost entirely in terms of size of flowers thrown off
N. Tabacum var. macrophylla	all buds up to anthesis.
N. Tabacum "Maryland"	all flowers up to 4 or 5 days past anthesis.
F_1 H154	all buds up to opening of corolla.
F_1 H36	all buds and flowers.
F_1 H179	all buds and flowers.
N. glauca	young buds.
N. rustica var.?	buds up to anthesis.
N. rustica var.?	buds, flowers, and fruits.
N. Bigelovii var. Wallacei	no abscission.
N. Bigelovii "Pomo"	no abscission.
N. quadrivalvis	no abscission.
N. multivalvis	no abscission.
N. Sanderae	buds up to anthesis.
N. suaveolens	buds up to anthesis.
N. plumbaginifolia	buds up to opening of the corolla.
Solanum umbelliferum	small buds.
S. jasminioides	buds and flowers.
S. verbascifolium	no abscission.
S. nigrum	small buds.
Iochroma tuberosa	no abscission.
Cestrum fasciculatum	buds and flowers.
Lycopersicum esculentum var. pyriforme	no abscission.
L. esculentum var. vulgare	small buds and occasional flowers.
Petunia hybrida	no abscission.
Salpiglossis sinuata	no abscission.
Datura sanguineum	buds and flowers.
Salpichrora rhomboidea	no abscission.
Lycium australis	no abscission.

As might be expected, most of these varieties react to laboratory air in the same manner that they do to illuminating gas. In the case of laboratory air a longer time and higher temperature is generally required before the reaction occurs. All the species, with the exception of those which throw off only young buds, detach most of their flowers when left in laboratory air overnight. If a window or two is left open, allowing fresh aid to enter and at the same time lowering the temperature, no abscission occurs.

It was found that several of the species recorded above, in which no abscission or very little abscission occurred, detached more flowers when a larger percentage of gas was used or when subjected to 1.5 per cent gas for a longer time. Thus, both varieties of *Lycopersicum*

esculentum, Iochroma tuberosa, Solanum nigrum, and *S. verbascifolium,* upon subjection to 3 per cent illuminating gas for twenty hours, throw off all flowers up to those two or three days past anthesis. No abscission occurred, however, in any concentration of gas, in *Nicotiana Bigelovii, N. quadrivalvis, N. multivalvis, Lycium australis, Petunia hybrida, Salpiglossis sinuata,* or *Salpichrora rhomboidea.*

A peculiar condition exists in *Solanum umbelliferum,* which throws off buds in the illuminating gas but never under any conditions, including temperature or the presence of narcotic vapors, throws off flowers in which the corolla has fully opened. A corresponding condition seems to exist in *Nicotiana Tabacum* var. *macrophylla,* F_1 H154, *N. Sanderae, N. rustica* var. *brasilia,* and in one other variety of *N. rustica,* all of which seldom under any conditions detach fully opened flowers, although flowers up to that stage are freely abscissed. Thus there seems to be, in certain species and at about the time of the opening of the corolla, a sudden increase in resistance to the external stimulus which is causing abscission. In other species this sudden increase in resistance does not take place, abscission commonly occurring at any stage in the development of the flower or fruit and the increase in resistance taking place very gradually. In addition, there seems to be an intergradation of forms between those in which the increase in resistance takes place suddenly and those in which it takes place gradually.

The next subject to be taken up is a consideration of experiments 5, 6, 7, 8, and 9 on the induction of abscission in small isolated pieces of the pedicel. The main purpose of devising these experiments was to throw some light, if possible, on the direct or indirect action of the external factor in causing "spontaneous" abscission. The pedicel of F_1 H179 was again chosen as material for the following experiments,

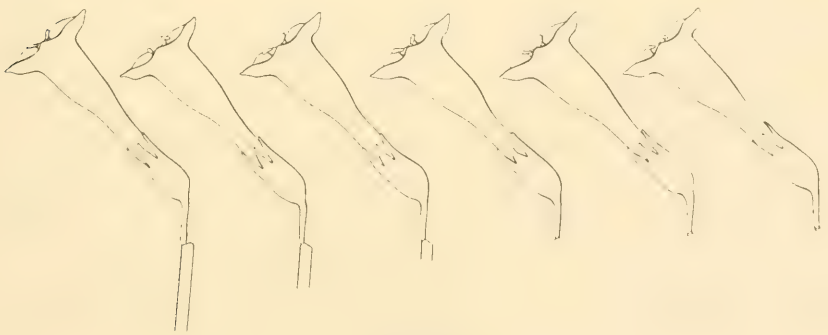

Fig. 9

largely because of the ease and regularity with which abscission is induced in this hybrid by sudden changes in the external environment.

Experiment 5.—This experiment was devised to discover the effect of reducing the volume of material proximal to the separation layer on the abscission of flowers of *Nicotiana* as induced by illuminating gas. Two series of flowers were cut as in figure 9. In the last two flowers represented on the right the cut was made less than 0.5 mm. from the separation layer. These flowers were then rolled in damp filter paper and left in 1.5 per cent illuminating gas overnight. After fifteen hours, abscission had occurred in all the flowers except the one represented on the extreme right in the figure. Abscission had occurred in one flower in which the cut had been made less than 0.5 mm. from the separation layer. The control to this experiment showed that abscission does not occur for several days in a series of flowers cut as in figure 9 and kept under normal conditions.

Experiment 6.—This experiment was devised to show the effect upon abscission of reducing the volume of material distal as well as proximal to the separation layer. In this case the flowers were cut off at varying distances from the separation layer, making the series shown in figure 10. The last two pieces on the right in this series were cut less than 0.5 mm. on each side of the separation layer so that the total length of the pieces was not much above 1 mm. In this experiment and in similar ones which follow it was necessary to keep

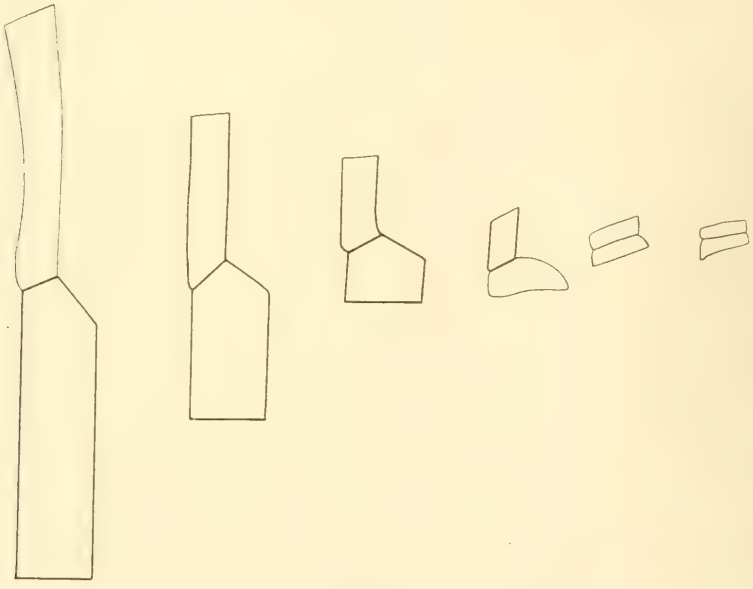

Fig. 10

the material moist. This was accomplished in various ways, but the best method was found to consist in placing the pieces on a long strip of filter paper one end of which rested in water. In this experiment abscission occurred after ten hours subjection to 1.5 per cent illuminating gas in all except the two pieces represented in the extreme right of figure 10. Abscission here took place in several pieces ranging from 1 mm. to 2 mm. in length. A microscopic examination of the separation surfaces indicated that the process of abscission corresponded entirely with normal abscission as it occurs in plants in the field. Experiments made in a similar manner upon *N. Tabacum* "Maryland" and *Lycopersicum* gave similar results. In the control, which consisted in keeping pieces of the pedicel as shown in figure 10 under normal atmospheric conditions, abscission occurred after about twenty hours, evidently as the result of no other stimulus than that caused through cutting off the flower by severing the pedicel. The reaction in the control, however, is much slower than in the case in which the added effect of the illuminating gas is operative, indicating that the latter factor, although it here serves merely to hasten the abscission process, has an effect of some kind on the tissues at the base of the pedicel.

Following these two experiments, a number of attempts were made in the same way to induce abscission in longitudinal free-hand sections of the pedicel cut for microscopical examination. It was soon discovered that the abscission process could be induced in the separation zone in thick longitudinal sections of the pedicel by subjecting them to high percentage (5 to 7 per cent) of illuminating gas. Cell separation in cross-sections through the separation zone could not be induced by any means at hand. The following experiments give more detailed results in this connection.

Experiment 7.—In this experiment, median, longitudinal sections of varying thickness were cut through the pedicels so that the plane of the sections corresponded with the plane formed by both the pedicel and the main axis of the inflorescence. These sections were subjected to 7 per cent illuminating gas, care being taken to keep them moist, but not submerged, throughout the entire experiment. The best arrangement was found to be one in which the sections rested in a thin film of water on one side but were exposed to the air on the other. After several hours in the 7 per cent illuminating gas, abscission started in the thicker sections but not in the thinner ones. The extent to which abscission proceeded depended upon the thickness of

the section. Abscission became complete in sections 0.3 mm. or more in thickness, the separation taking place in such a way that a slight bending or pulling motion sufficient to break the tracheæ divided the section into equal halves. In thinner sections, ranging from 0.3 mm. to 0.17 mm., abscission starts in the normal position but does not proceed to completion, the extent to which the process takes place depending, as has been said, upon the thickness of the section. In sections much below 0.17 mm. no signs of abscission appear. Also, if the thicker sections are shortened in length to any considerable extent by cutting off portions of the tissues from either side of the separation layer, abscission will not occur.

The process of abscission as it occurs in these sections corresponds exactly to the process in an entire pedicel. Cell separation starts independently in the pith and in the cortex, appearing first in that part of the cortex corresponding to the ventral region of the pedicel where, it will be remembered, abscission starts in the entire flower. When mounting the sections on an object slide for microscopical examination, the isolated cells in the pith lie in position but can be easily washed out with a small jet of water. In the cortex a break soon appears in the epidermis as the result of manipulation in mounting and a cavity is formed at that point as the result of the isolated cells of the cortex floating out in the water.

Experiment 7 was repeated in the case of *Datura* with similar results, except that in this case abscission was more active since it involved more cells, a situation which one might be led to expect because of the differences between the two species in the normal abscission of entire flowers. It will be remembered that the separation cells of the cortex in *Datura* are in no way distinguishable from other cortical cells; yet even in these sections separation occurs in a definitely predetermined position corresponding entirely with the position in abscission of the entire flower. It was even noticed that abscission started in these sections in the same tissues and in the same manner as in normal floral abscission.

After the thickness of the sections best adapted to obtaining results had been determined, the following experiment was performed on sections cut from different parts of the pedicel.

Experiment 8.—In this experiment a series of longitudinal sections of the pedicel were cut so that the plane of the sections was at right angles to that of the sections cut in Experiment 7. The first section was tangential, on the ventral side of the pedicel, and contained only the epidermis and a few tiers of cortical cells. Section 2 was also

tangential but contained a few tracheæ on one surface. Section 3 was more or less radial, containing two strands of vascular tissue on either side. Sections 4 and 5 were similar to sections 1 and 2. On subjecting these sections to illuminating gas it was noticed that abscission started first in sections 1, 2, and 3, appearing last in sections 4 and 5. This result is exactly parallel with the process as it occurs in normal abscission, where the process starts first in the ventral cortex and in the pith.

In passing, mention might be made of the peculiar reaction of the tangential sections 2 and 4, which were made up almost entirely of cortical cells with a few vascular elements on one side. When abscission occurred in these sections, a bending or bowing of the section was always noticed. This bending was always such that the tracheal tissue was on the concave side, as if the cells of the cortex had undergone considerable expansion while the cells of the vascular tissue retained their original size. From the work of Richter and others, it may be expected that subjection of portions of plant tissues to illuminating gas would cause an increase in turgor in the cells concerned. Thus, it is probable that the bending of the sections, as described above, is due to the increase in turgor of the cortical cells caused by the narcotic effect of the illuminating gas. The extent of the bending was such that most of the cells in the cortex as well as the separation cells must have been involved in the process. On repeating the above experiment with *Datura,* a similar bending of the tangential sections was even more pronounced than in *Nicotiana.*

Experiment 9.—As mentioned above, efforts to induce abscission failed in thin sections. The sections in Experiment 9 were cut so that they were thin in the separation layer but thick on either side. Both surfaces of these sections were thus cut slightly concave so that the sections were thickest at the ends and thinnest in the middle, where the separation zone was located. The sections were then subjected to 7 per cent illuminating gas as in Experiment 7. It was not possible to cut very thin free-hand sections of the shape described, but it was demonstrated without a doubt that abscission occurred in sections of this peculiar shape which were thinner in the separation zone than those in Experiment 7 where abscission had failed to occur.

Certain conclusions which can be drawn from experiments 5, 6, 7, 8, and 9 are given below.

1. Abscission can be induced by allowing the external factor to act directly upon the cells in the vicinity of the separation zone (Expts. 6, 7, and 8).

2. Abscission induced by the above methods in isolated pieces must be independent of transportation of material from the rest of the plant.

3. The fact that abscission cannot be induced in thick cross-sections of the separation zone shows that cell separation cannot be induced by the action of the external factor directly on the separation cells.

4. It is necessary that a certain proportion of the tissues of the pedicel be in intercellular connection with the cells of the separation zone before cell separation will occur, but this proportion is surprisingly small (Expts. 7, 8, and 9).

5. There is evidently increase in turgor in all the cortical cells of the pedicel during abscission induced by the above method (Expt. 8).

2. Action of Acids on the Separation Cells of *Nicotiana*

Under this heading a description will be given of the effect of mineral acids on small isolated pieces such as were used in experiments 6, 7, 8, and 9. It was stated above (page 364) that by the use of two mineral acids together with several stains, no chemical difference could be detected between the cell walls of the separation cells and those of normal cortical cells. The present work represents an attempt to determine, by experimental means and by watching through the microscope the action of acids on cell walls, whether the cell membranes of the separation cells are more subject to hydrolysis than those of normal cortical cells.

Experiment 10.—Small pieces of the pedicel were prepared as in figure 10. These pieces were boiled for one or two minutes in 4 per cent hydrochloric acid and then washed in water. Upon examination it was found that the pieces could be separated into halves through the separation zone by a slight pulling or bending motion. Microscopic examination of the separation surfaces showed that the break through the cells of the separation zone had taken place along the plane of the middle lamellae of their walls. This same type of separation was brought about without boiling when 10 per cent nitric or hydrochloric acid was allowed to act on the pedicels for approximately five minutes. When longitudinal sections are used in place of entire pedicles, the same results are obtained but much more rapidly. It was also noticed that separation under these latter conditions takes place more quickly in younger pedicels than in older ones. In the pedicels of fully developed fruits no separation could be induced, but in those of

immature fruits separation occurred in the cortex but failed to take place within the vascular cylinder.

Experiment 10 at first glance would seem to indicate that the cell walls of the separation cells are more subject to hydrolysis than normal cortical cells. Another interpretation is possible, however. Actual separation which takes place through the separation zone may be due to the fact that the cells in this zone are small and have a tendency to be isodiametric, whereas the remaining cells of the cortex are larger and are elongated parallel to the long axis of the pedicel. Hydrolysis of the cell walls may go on with equal rapidity in all the cortical cells at the base of the pedicel, yet upon bending or pulling separation may take place through the region of isodiametric cells because of the interlocking of the elongated cells in the rest of the cortex. An attempt was made to gain further evidence on this point by observing through the microscope the action of acids on the cell walls of the tissues concerned. When the action of the acids is thus observed, the walls are seen to soften and to swell to two or three times their normal thickness. This effect is all the more noticeable if the walls initially are comparatively thick. Now, since the cells of the separation zone are small and somewhat collenchymatous, or at least have thicker walls than normal cortical cells, the process of swelling in the cell wall is most conspicuous in that region. Indeed, hardly any swelling can be perceived as a result of the acid treatment in the cell walls of normal parenchyma cells of the cortex. However, when a form such as *Lycopersicum* is examined in which there is a distinct layer of collenchyma beneath the epidermis for the entire length of the pedicel, this collenchyma appears to be affected at the same time and in the same manner as the cells of the separation zone of *Nicotiana*. Also in *Nicotiana* there seems to be a certain amount of similarity in reaction to acids between the smaller cells of the cortex just beneath the epidermis and those of the separation zone. The conclusion can thus be drawn that the cell walls of the separation cells are no more readily hydrolyzed than those of normal collenchymatous tissues. Of course, the fact still remains that the collenchyma of the cortex may be more subject to hydrolysis than the cortical parenchyma. Now the small cells of the separation zone not only extend across the base of the pedicel but also spread throughout the general region at the base of that organ; it was therefore noticed that the swelling of cell walls was by no means confined to cells of the separation layer but was more or less prominent throughout the whole general region at the base of the pedicel.

The general results of these observations are in a sense negative and seem to indicate that the walls of the separation cells are no more subject to hydrolysis than the walls on either side. This, of course, does not preclude the possibility that a difference exists which is too slight to be detected. It appears, however, that the general region at the base of the pedicel may be more subject to hydrolysis than the more distant portions.

3. Induction by Mechanical Injury

The results of experiments on the induction of abscission by mechanical injury are recorded in tables 2, 3, 4, and 5, which have already been considered under the heading, "Time of Abscission" (page 384).

Several facts of interest brought out by table 2, which deals with *Nicotiana Langsdorffii* var. *grandiflora*, are summarized below.

1. It appears that removal of or injury to the capsule does not cause abscission in mature fruits (table 2, *a*, *b*, and *h;* table 3, *c* and *d*). The same types of injury generally do cause abscission in immature fruits.

2. It seems that a transverse cut completely through the flower at the distal end of the calyx causes abscission only in buds or flowers near anthesis (table 2, *c*). It appears, however, that such a cut proximal to the distal end of the calyx causes abscission in flowers several days past anthesis as well as in buds (table 2, *a*, *b*).

3. Removal of the entire calyx causes fall in very young buds only (table 2, *d*).

4. It seems that slitting both the corolla and calyx longitudinally on both sides from tip to base does not induce abscission even in young buds (table 2, *e*).

5. Entire removal of the style or stamens causes fall only in young buds (table 2, *f* and *g*).

6. It appears that injuries to the pedicel do not cause abscission, provided the flower is not entirely cut away (table 2, *i*). Just here it is worth mentioning that two of the pedicels cut transversely as recorded in table 2, *i*, were cut so deep that the flowers bent over and hung only by a few vascular strands and cortical cells. The wound healed over, however, and the two flowers matured with the rest.

7. It is evident that injuries which reach the ovary are much more effective in causing abscission than injuries affecting the other parts of the flower (table 2, *b* and *e*).

8. Fertilization has no influence whatever in preventing abscission when the latter is induced by a transverse cut completely through the flower at the base or middle of the calyx (table 3, *c* and *d*).

9. Certain types of injury, such as entire removal of the calyx and stamens or removal of the entire calyx and half the corolla, evidently cause abscission only by preventing fertilization (table 3, *a* and *b*).

Taking up now the results given in table 4, which dealt with F_1 H179, it will be seen that this hybrid is more sensitive to injury than is *N. Langsdorffii*. Nevertheless, it is very plain that the general conclusions announced above for this latter species hold for F_1 H179 also. There follows a partial summary of the results in table 4 and a comparison of these results with those obtained in the experiments on *N. Langsdorffii*.

1. It seems that removal of the calyx causes fall of much larger buds than in *N. Langsdorffii* (table 4, *d*).

2. F_1 H179 is evidently much more sensitive in its abscission reaction to a transverse cut through the flower at the middle of the calyx than *N. Langsdorffii* (table 4, *a*).

3. It would seem that slitting the calyx and corolla even to the extent of dividing these organs into four longitudinal strips does not, as a general rule, cause abscission. Such an injury does cause abscission only in extremely small buds (table 4, *g*).

4. It appears that puncturing the calyx, corolla and ovary so that a hole is formed about 2 mm. in diameter in the latter organ causes fall in flowers of all sizes up to two or three days past anthesis (table 4, *h*). Since it is evident that such a hole through the calyx and corolla alone would not cause abscission (table 4, *g*), abscission in this case must be induced by injury to the ovary.

5. It is evident that a slit completely through the pedicel for its entire length fails to cause fall in buds or open flowers, but where an effort is made to destroy completely the connection between the flower and stem abscission will occur (table 4, *i*).

6. Removal of the style or stamens, as a general rule, causes fall only in young buds, but removal of the former organ is probably more effective in causing flower-fall than removal of the stamens (table 4, *e* and *f*). On the other hand, where half the corolla is removed along with the stamens fall occurs in larger buds than where only the latter organs are removed (table 4, *b*).

7. Removal of only half the corolla apparently does not induce abscission (table 4, *c*).

8. Mature capsules of F_1 H179 are apparently more sensitive to injury than those of *N. Langsdorffii* (table 4, *j*).

The table dealing with the experiments on *Lycopersicum* indicates that flowers of this genus are remarkably resistant to injury, fall occurring only as the result of stimulation when the ovary is injured (table 5, *c* and *d*). Since a large number of tomato flowers are normally abscissed from the different inflorescences on a plant, the several exceptions to the above statement noted in the table probably demonstrate to what extent the normal physiological condition of the plant affects the matter. It seems to be the opinion of most gardeners who are familiar with the tomato plant that floral abscission in this species is more dependent upon soil conditions than upon injury or sudden changes in climatic conditions. It would seem, however, that injuries to very young fruits normally cause fall, but in this case a stage of development is soon reached at which injury to the berry has no effect in inducing abscission (table 5, *f*).

Taking the general results of all the experiments into consideration, it is seen, in the first place, that where injury of a certain type causes fall, a stage of development of the flower is soon reached beyond which the injury no longer causes fall. The increase in resistance to the stimulus of mechanical injury takes place gradually in the species investigated, but some of the species are much more resistant than others. In the second place, injuries to the ovary generally cause flower-fall. Thirdly, whether or not flower-fall occurs as a result of injury to other flower parts depends in some way upon the quantity of material removed. Fourthly, injury to the pedicel does not cause abscission unless it breaks entirely the cellular connection between flower and stem. Lastly, it is improbable that fall induced by injury is due to checking the transpiration stream, since injury to the ovary could have no such effect. Also, a cut across the pedicel so that the flower hangs by only a few tracheæ must check transpiration from the flower considerably, yet in this case no abscission occurs.

It was suggested by Bequerel that injury might cause abscission by checking the transpiration stream which passes up through the pedicel. Considerable doubt has already been cast on this point in the above discussion. In order to throw more light on this question the following experiment was performed in an effort to determine whether checking the transpiration stream of itself and unaccompanied by mechanical injury would cause abscission.

Experiment 12.—As a means of checking transpiration from the flower a coating of paraffin seemed desirable because it hardens

quickly, thus permitting several coats to be applied. It was doubtful whether other substances, such as lard, cocoa butter or vaseline, which might have been used, would not have been prevented from completely covering the flower in one coating by the presence of numerous hairs and glandular fluid on the calyx. In this experiment flowers were immersed in melted paraffin to within a millimeter of the separation zone and allowed to stand in water under normal atmospheric conditions. As a test for abscission, the shoot was shaken or individual flowers tapped from time to time. It was found that several *Nicotiana* varieties and hybrids differed in their reaction to this treatment as they did in their reaction to illuminating gas. In *N. Tabacum* "Maryland," for example, paraffining the flowers failed to cause abscission for six days, at the end of which time the flowers began to fall, as did those of the control. Some varieties, however, under such treatment, throw off buds at the end of twenty-four hours, but open flowers of the same varieties are never shed. Whether or not the buds fell in these varieties depended largely on the temperature, at lower temperatures no fall occurring. Also, in cases where abscission of buds did occur it was evident that something was actually impeding the process; none of the white substance formed by the isolated cells was seen at the base of the pedicel and the buds had to be shaken or tapped quite severely before they fell.

The results of Experiment 12 and the various observations on the induction of abscission by mechanical injury render it extremely unlikely that checking the transpiration stream is ever a direct cause of abscission. The few cases recorded above in which such a condition seems to cause abscission can be better explained by the action of some other factor than that of interference with transpiration.

In connection with these experiments upon the effect of checking transpiration the results of Lloyd and Balls on the effect of root pruning, etc., in cotton must be mentioned. It was found that a premature shedding of flowers and young bolls followed root pruning and further that, in general, there is a relation between boll-shedding and the rise and fall of the water-table. Proof positive is not supplied that root pruning causes fall of flowers by reducing the water supply of the plant body, and any number of other factors may enter in after such mutilation to bring about, in part at least, such a result. Experiments reported in the present paper seem to leave no doubt that, in *Nicotiana* at least, temperature is a more important factor in controlling abscission than water supply.

4. The Ability of Certain Species to Throw off Pedicels from which All the Floral Organs Have Been Removed, as Related to the Induction of Abscission by Mechanical Injury

It was soon noticed in the experiments that all plants of a species in which floral abscission occurs throw off the remains of the pedicel when this organ is severed at any point distal to the separation layer. If after such an operation no abscission occurs, it can be safely concluded that floral abscission never occurs in that species. *Petunia hybrida, Salpiglossis sinuata, Salpichrora rhomboidea,* and *Lycium australis* are the only species of the list in table 6 which do not abscise flowerless pedicels in this way. *Nicotiana Bigelovii, N. quadrivalvis,* and *N. multivalvis* occasionally do not throw off pedicels under such conditions. The reaction time in cases where the last three species do abscise severed pedicels is very slow (four to fourteen days).

Turning now to the relation of these observations to the induction of abscission by mechanical injury, it is first necessary to recall the controls used in Experiments 5 and 6 (cf. pages 399 and 400). A further consideration of the reaction of these controls will suggest that mechanical injury can induce abscission by the action of the stimulus directly on the cells in the vicinity of the separation zone. The control used in Experiment 5, it will be remembered, showed that abscission does not occur under normal conditions in a series of flowers cut as in figure 9. From the control used in Experiment 6 it is evident that merely cutting off the flower at varying distances from the separation layer, forming pieces as represented in figure 10, causes abscission to occur, evidently as the result of no other stimulus than that of severing the pedicel. Now, if the cut be made through the pedicel at a point approximately 1 mm. distal to the separation layer in flowers, as represented on the extreme right of figure 9, abscission will occur in the remaining piece, which is now scarcely 2 mm. in length. It is evident that the stimulus caused by severing the pedicel must act directly on the cells in close proximity to the separation zone. Practically the same results are obtained when the transverse cut is made through the base or middle of the calyx. There is no reason to suppose that the stimulus set up by cutting through the flower near the base or middle of the calyx differs in any fashion from that offered by a cut severing only the pedicel.

Several interesting conclusions are brought out by an examination of the above facts. In the first place, the abscission of the remains of severed pedicels is probably independent of the transportation of materials from the rest of the plant to the separation zone. It may result from the action of the stimulus directly on the cells in the vicinity of the separation layer and is, therefore, largely independent of such physiological processes as transpiration which might conceivably enter in. In the second place, abscission induced by mechanical injury is probably of the same nature as that of severed pedicels and therefore probably results from the action of the stimulus directly on the cells in immediate proximity to the separation layer.

SUMMARY

The final summary of results given below is presented under several headings corresponding to those of the main body of the paper. Unless otherwise stated, the results given may be taken as applying to all the species of the Solanaceae in which abscission was found to occur. First is presented a complete list of the species which were investigated, indicating by 1 those in which floral abscission never occurs, by 2 those in which it very seldom occurs, and by 3 those which were actually examined microscopically to determine the histological structure of the separation zone and the method of abscission.

3 N. Tabacum var. macrophylla
3 N. sylvestris
3 N. Tabacum "Maryland"
3 F_1H154 (N. sylvestris × N. Tab. var. macrophylla)
3 F_1H179 (N. sylvestris × N. Tabacum "Cuba")
3 F_1H36 (N. sylvestris × N. Tab. var. angustifolia)
N. glauca
3 N. rustica (2 varieties—not brasilia)
2, 3 N. Bigelovii (3 varieties)
2 N. quadrivalvis (2 varieties)
2 N. multivalvis
N. Sanderae
N. rustica var. brasilia
N. suaveolens
3 Solanum umbelliferum
S. tuberosum
S. jasminioides
3 S. verbascifolium
S. nigrum
2, 3 Iochroma tuberosa
3 Cestrum fasciculatum
Lycopersicum esculentum var. vulgare
3 L. esculentum var. pyriforme
1, 3 Petunia hybrida
1, 3 Salpiglossis sinuata
3 Datura sanguineum
1 Salpichrora rhomboidea
1 Lycium australis

Histology and Cytology of the Pedicel

1. The separation layer arises in all the species listed above, except *Lycopersicum* and *Solanum tuberosum*, at or near the base of the pedicel. In the latter two species the layer is located near the middle of the pedicel, but even in these cases, if one considers the pedicel to be composed of two internodes, the layer occurs at the base of the most distal internode.

2. The separation layer is preformed, ready to function at any stage in the development of the flower and represents (cf. Kubart's first type, page 350) a portion of the primary meristem which has retained some of its originally active condition.

3. In all the species except *Datura* the separation cells are characterized by their small size, isodiametric shape, large amount of protoplasm and somewhat collenchymatous appearance. A study of the early histological development of the pedicel indicates that the small size of the separation cells does not necessarily bear any relation to abscission. This statement is supported by the fact that in *Datura* there is absolutely no visible difference between the separation cells and any other cells of the pedicel.

4. Various tests with stains, acids, and alkalis fail to indicate any chemical difference between the cell walls of the separation cells and the walls of neighboring cortical cells which do not separate. However, the middle lamellae of cell walls in the general region at the base of the pedicel seem somewhat more easily hydrolysed by acids than in the more distal portions.

5. A study of the early histological development of the pedicel in *Nicotiana* and *Lycopersicum* shows that the grooves near which the separation zone arises do not necessarily bear any relation to abscission. The grooves are formed because, in the development of the pedicel, certain cells do not increase in size so fast as the neighboring cells on either the proximal or distal side.

6. The development of mechanical tissue in the pedicel of *Nicotiana* continues through the separation layer, thus frequently holding the fruit on the plant in spite of the fact that abscission commonly occurs in the cortex. In most of the berry-forming species of the Solanaceae this mechanical tissue does not become continuous through the separation layer and thus offers no impediment to fall when abscission occurs in that region.

The Process of Abscission

1. The process of abscission conforms to the usual type, which involves the separation of cells along the plane of the middle lamella of the cell wall separating them.

2. No cell divisions or elongations were observed to accompany abscission.

3. All the cells across the pedicel in the region of the separation layer take part in separation except the tracheæ and cuticle, which must be broken mechanically. The total number of cells which may be involved is greater in some species than in others. This number may also vary in the same species because of changes in the external conditions.

4. Cell separation is brought about by the hydrolysis and consequent dissolution of the middle lamella (primary cell membrane) or perhaps both the primary and, in part, secondary cell membranes. The agency active in the hydrolysis of the cell membranes is probably an enzyme.

5. An increase in cell turgor frequently occurs during abscission, but probably serves merely to hasten and facilitate the process. Most of the frequently observed expansion and the turgid appearance of the separation cells during abscission are probably due to the natural release of pressure caused by the dissolution of the middle lamellae.

6: Abscission of the style and corolla in *Nicotiana* and *Datura* resembles, to a large extent, abscission of the flower.

Time of Abscission

1. The length of time between anthesis and normal flower-fall due to lack of fertilization differs among the varieties of *Nicotiana*. This variation was found to range between an average of five to eighteen days in some fifteen species and varieties of *Nicotiana*. A much smaller range of variation (0.7 to four days, with the largest frequency in the three day group) was noted for the time between anthesis and fall of the corolla after pollination.

2. The stimulation of the stylar tissues by the growth of the pollen tubes tends to shorten the time between anthesis and fall of the corolla, this effect being independent of fertilization. Such stimulation of the stylar tissues has no appreciable effect upon floral abscission.

3. Floral abscission occurs in F_1 H179 seven hours after subjecting shoots of the plant to 1.5 per cent illuminating gas at a temperature

of 19° C. It occurs in *Nicotiana Tabacum* "Maryland" in eight hours under the same conditions. The actual time involved in the process of cell separation in the above-mentioned cases lies within thirty to forty minutes in the hybrid and within forty-five to sixty minutes in the *Tabacum* variety. Normal abscission in these forms is much slower

4. The length of the reaction time in cases of flower-fall due to mechanical injury shows that this length of time depends more on the age of the flower than on the type of injury.

5. Temperature is the most important conditioning factor in estimates of the time of abscission.

Experimental Induction of Abscission

1. Floral abscission is induced, in a large number of the species investigated, by illuminating gas or laboratory air. The increase in resistance to abscission stimulated in this manner takes place suddenly in some species, since abscission will not occur after the opening of the corolla. In other species this condition does not exist.

2. It is possible to induce the process of abscission with illuminating gas in small isolated pieces of the pedicels or in longitudinal sections of the pedicel cut free-hand from fresh material.

3. Abscission in *Nicotiana* and *Lycopersicum* is induced by certain types of severe injury and not by others. Injury to the ovary seems more effective in causing abscission than injury to other parts of the flower. In the case of these other flower parts, it seems necessary that a certain amount of tissue be actually removed or destroyed before fall occurs. Injury to the pedicel does not cause abscission unless it breaks entirely the connection between floral organs and stem. Flower-fall in *Lycopersicum* is not readily induced by injury. Floral abscission in this genus is more dependent upon physiological conditions brought on by abnormal soil conditions.

4. Experiments on the induction of abscission in small isolated pieces and in flowers with only a small portion of the stem proximal to the separation layer attached indicate that the stimulus produced by the action of external factors such as illuminating gas and mechanical injury can cause abscission by acting directly on the cells in close proximity to the separation zone. The action of external factors is thus largely independent of such physiological processes as transpiration which might enter in. This statement is supported by experiments which show that abscission is not necessarily induced by checking transpiration from the flower.

CONCLUSION

It is proposed in what follows to take up consideration of such phenomena in connection with abscission as are still but slightly understood. One of the most perplexing of these is undoubtedly the definitely predetermined location of the separation layer when no morphological and sometimes no physiological (*Datura*) difference can be detected between the cells that separate and those that do not. There need be no doubt, however, that such a difference does exist and that a sufficient refinement of technique will serve to detect it.

In considering this matter further it may be recalled that the separation layer in axial abscission is located at or near the base of an internode. There is undoubtedly some connection between this fact and the fact that the cells most active physiologically are often found in this region. The growth of an internode may be brought about by the action of an intercalary meristem located at the base of the organ and a meristem so located in some cases retains its original activity in the mature internode. Now it is well known that the walls of young active cells are more readily subject to hydrolysis than the walls of older cells, because of the fact that the former contain more water. If we assume, then, that the internode is a metabolic gradient with the most active cells at the base, it would be expected that the walls of these cells would be more subject to hydrolysis than any other cells of the internode. If some hydrolysing agency becomes active throughout the pedicel, it might be expected that the walls of the cells at the base of the internode would react first, causing their separation and thus cutting off the flower or internode. By assuming in this way that separation always takes place through the most active cells of the internode it seems possible to explain the predetermined location of the separation layer.

There is undoubtedly some connection between the above problem and the fact that some plants must perfect a separation layer before detachment can take place. In such cases the tissues at the base of the organ are too old for separation. The same stimulus which causes abscission in some species causes a renewal of activity at the basal region of an organ, resulting in cell divisions and new cells. These new cells may, under a continuation of the stimulus, separate one from another.

Another perplexing problem, which also includes many subsidiary problems, relates to the exact course taken by the stimuli in causing

abscission. Experiments described in the present paper have indicated that this course may be direct as well as indirect. Assuming for the present that some of the factors bringing about abscission always act directly while others act indirectly, we might classify the general factors operative in the case of the Solanaceae as follows:

Direct	1. Narcotic vapors.
	2. Injury to floral organs.
	3. Sudden rise in temperature.
	4. Lack of fertilization.
Indirect	5. Changes in soil conditions.
	6. Factors evident in normal physiological development.

The direct factors act directly on the cells at the base of the pedicel and consequently the reaction time must be comparatively rapid. The indirect factors act indirectly through the general physiological condition, which in turn furnishes the direct stimulus for cell separation. In the latter case the reaction time must, as a general rule, be slow. The nature of factors under 6 are most difficult to understand. An example of the action of these factors would be given in those cases where most of the flowers of an inflorescence are normally abscissed leaving only one or two to continue development, and in those species which abscise male flowers after anthesis.

A further analysis of the course of the abscission reaction introduces another unsettled problem—the nature of the agency which is directly responsible for the dissolution of the middle lamella. It has been pointed out before that an enzymatic body of some kind is probably involved. The following discussion brings out certain facts which it is necessary to take into consideration when speculating as to the nature of this supposed enzyme. The activity of the enzymatic body must be subject to both internal and external conditions. The enzymatic material must also be extremely sensitive to slight changes in the normal environment. It must be continually present in the cells of the separation zone and ready at any moment to react to such changes in the environment. A comparison of several species in regard to their abscission reactions to the factors listed above indicates that this supposed enzyme must be more sensitive in some species than in others. Indeed, in certain species in which no abscission occurs the enzyme must be absent from the region of the separation zone or entirely inactive. Finally, it seems necessary to assume that in certain species the action of the enzyme is suddenly inhibited at about the time of the opening of the corolla.

It has been noticed in all the experiments detailed above that older flowers are less subject to "spontaneous" abscission than younger ones. The transition line as to size or age beyond which no abscission occurs can not in most cases be definitely drawn; that is to say, the development of a resistance to stimuli takes place gradually. This is probably explained by the fact that cell walls gradually become less subject to hydrolysis with age. The celluloses and pectoses lose water with age and it is well known that these compounds are subject to hydrolysis in proportion to the amount of water they contain. In those cases where the increase in resistance to stimuli takes place suddenly it is necessary, as suggested above, to assume some kind of inhibitor of the enzymatic action.

The effect that pollination has in hastening abscission of the corolla is a subject which is related to the phenomena described by Fitting (1909) for orchids. The phenomena are as yet only slightly understood. The explanation seems to involve some relaying of stimulus from cell to cell. This is also involved in the explanation of floral abscission induced by injury to the ovary. These two cases and others indicate that in some instances, at least, abscission responses are related to tropistic responses as Fitting (1911) has suggested.

Finally, attention may be called to the fact that the most pressing need in connection with all the problems mentioned above is, in the first place, to establish by some experimental means a definite connection between some enzymatic body and the process of abscission and, in the second place, more definite knowledge as to the rôle which cell turgor plays in cell separation. Taking all the facts into consideration, it is evident that abscission is fundamentally a physiological problem, the crux of which lies, as in all such problems, in the biochemistry of the cell.

The studies reported upon above were carried on under the direction and supervision of Professor T. H. Goodspeed and I am under deep obligation to Professor F. E. Lloyd for many valuable suggestions both throughout the course of the experiments and during the preparation of this report of them.

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PLATE 49

Fig. 1. Base of pedicel of *Nicotiana* bud showing groove, separation zone, and process of abscission well under way in dorsal cortex.

Fig. 2. Portion of cortex in the separation layer of *Nicotiana* showing the bulging of the epidermis, one of the first signs of abscission.

Fig. 1

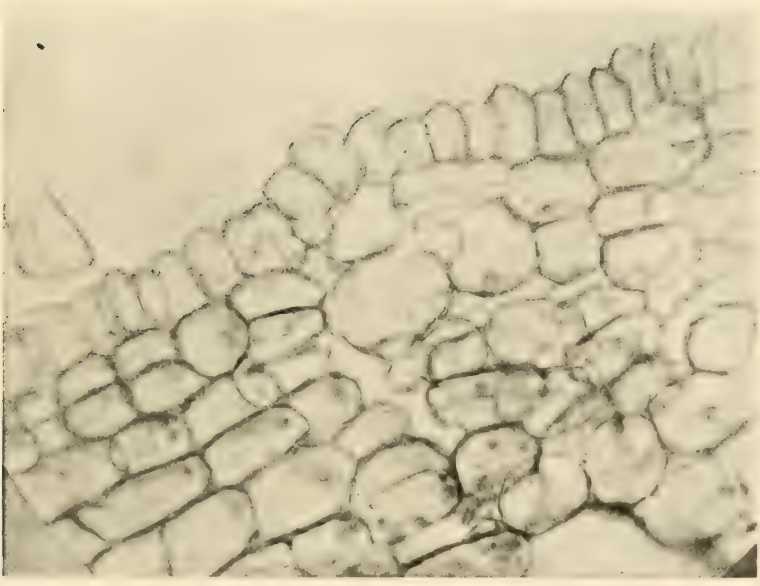

Fig. 2

PLATE 50

Fig. 1. Portion of the base of the pedicel of *Nicotiana* at a late stage in the process of abscission showing the independent origin of the process in the pith.

Fig. 2. Portion of the cortex in the separation layer of *Nicotiana* showing separating cells next to the vascular system.

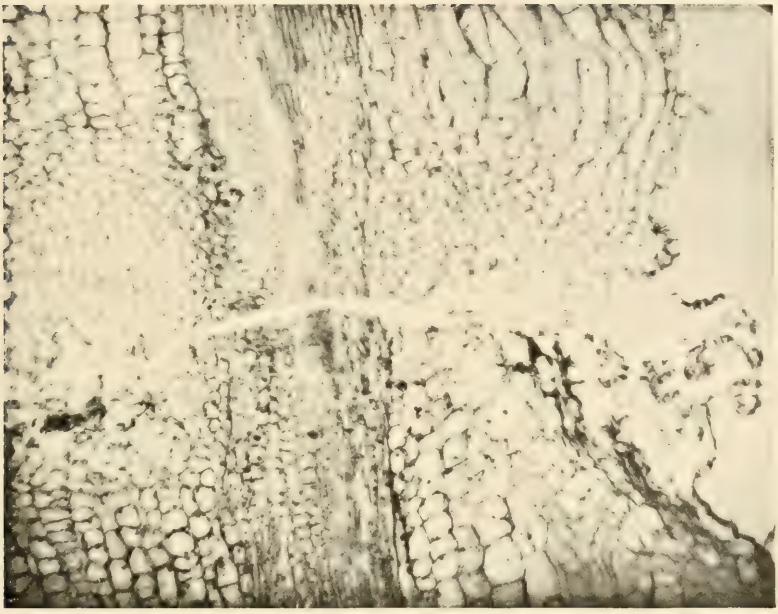

Fig. 1

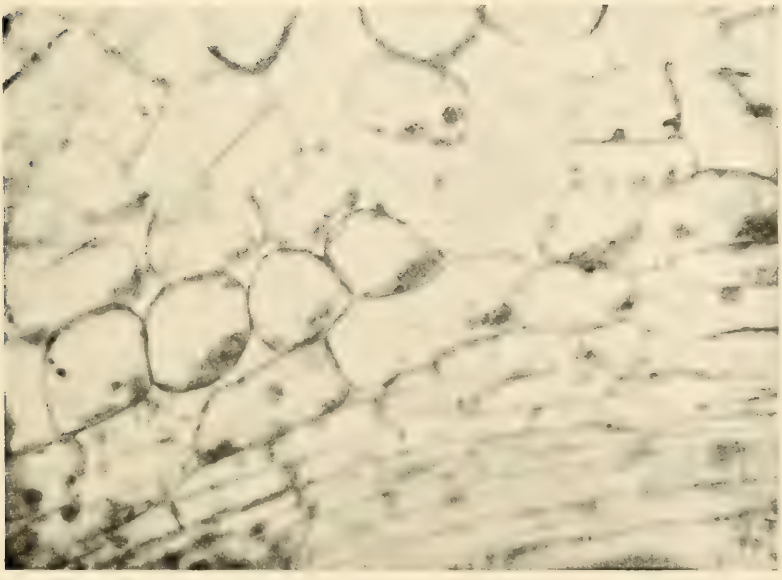

Fig. 2

PLATE 51

Portion of the separation layer of *Nicotiana* showing cells in the process of separation in the upper part of the section.

PLATE 52

Fig. 1. Portion of dorsal cortex near the groove in the pedicel of *Nicotiana*, showing the abscission process well under way.

Fig. 2. Group of isolated cells washed off from end of a freshly abscissed pedicel of *Nicotiana*.

Fig. 3. Single isolated cell showing the thinness of the remaining cell membrane.

Fig. 1

Fig. 2

Fig. 3

PLATE 53

Fig. 1. Portion of pedicel of *Lycopersicum*, showing groove and separation zone.

Fig. 2. Portion of cortex of pedicel of *Lycopersicum*, showing groove and abscission process fairly well along; cell separation first takes place between only two tiers of cells before spreading to others.

Fig. 1

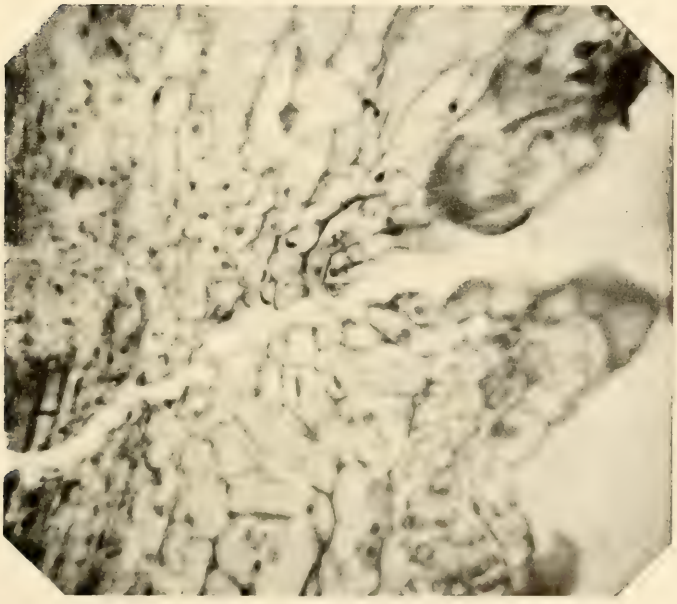

Fig. 2

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CONTROLLED POLLINATION IN *NICOTIANA*

BY
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In the course of the breeding experiments with *Nicotiana* carried on in the Botanical Garden of the University of California the question has arisen on a number of occasions and in a variety of connections as to the relation in this genus between the number of pollen grains applied to the stigma, the number of fertilizations accomplished and, finally, the number of seeds resulting. In the case of the F_1 *Tabacum-sylvestris* hybrids, for example, it has been shown that a few ovules capable of fertilization and the production of viable seeds are matured, and it has seemed possible that at least a corresponding percentage of normal pollen grains are produced. In field practice it is impossible, however, to obtain selfed seed of these hybrids even when the stigmas are artificially close, or self-pollinated with a considerable excess of pollen; the flowers thus treated falling after some days just as those allowed to pollinate themselves naturally under bag. In this connection it would be of interest to know whether in self-fertile species a relatively large excess of normal pollen is necessary to accomplish the fertilization of a relatively small number of ovules and whether the fertilization of three or four ovules is sufficient to inhibit the abscission which follows entire absence of pollination. Again, branching pollen tubes have been observed in a number of species of *Nicotiana* when the pollen was grown in artificial germinating media. If such branching occurs in the style and is accompanied by a division of male nuclei with the result that more than one ovule is fertilized by a single pollen grain, it would obviously be of importance in the interpretation of F_2 ratios, etc.[1] With these and other points in mind

[1] A cytological examination of a number of the branching pollen tubes gave some evidence that at least chromatin fragments were present in two or more of the branches. This fact might suggest that the abnormal germinating fluid of the pollen cultures was alone responsible for the branching and further resulted in a fragmentation of the initial nuclear material.

it was our intention to carry out an extensive series of experiments on a number of pure lines and hybrids of *Nicotiana* in which the number of pollen grains involved in each pollination was accurately known. Limitation of time and the lack of adequate greenhouse facilities have made it impossible to carry out our original plans. However, the results which have already been obtained seem of sufficient interest to warrant their publication at this time.

The particular results which are the subject of this paper were obtained in experiments upon six plants of *Nicotiana Langsdorffii* var. *grandiflora* (U. C. B. G. 107/08w). The pollination experiments were carried on in a greenhouse the average temperature of which was 30°C. It was known that the species used did not exhibit any traces of a parthenogenetic nature.

Flowering laterals were trimmed to a large bud and all leaves were removed. After careful castration these buds were bagged. When fully receptive a previously determined number of pollen grains was applied to the stigma and all pertinent data recorded on the copper wired pot-label which served to attach the bag to the plant. The paraffin bags were removed about two weeks after pollination.

The number of pollen grains used in the various pollinations was controlled in the following manner. A few grains were placed in the depression of a hanging-drop slide. Their number was determined and the preparation was covered with a cover slip and sealed with water. In the greenhouse the corolla of the flower was turned back, the cover slip removed, the slide carefully turned over and the pollen allowed to come into contact with the stigmatic surface covered with stigmatic secretion. Another count of the pollen grains remaining on the slide was then made to determine the number that had become attached to the stigma. Obviously defective grains were not considered in the pollen counts. The sources of error in any such method are obvious. However, the results given in the following table indicate that this method of obtaining controlled pollinations is approximately accurate. Other methods were tried but proved in every way less satisfactory than the one described above.

At maturity the ripened seed capsules were gathered and the number of seeds counted. It was possible in a few cases to determine the position of the seeds on the placental surfaces. None of the seed was tested as to its viability, but microscopic examination demonstrated the presence of normal embryos and endosperms. The results of some twenty-one controlled pollinations are given in the following table.

TABULATED RESULTS OF CONTROLLED POLLINATIONS IN *Nicotiana Langsdorffii* VAR. *grandiflora* (U. C. B. G. 107/08w)

Plant 17 was used as the pollen parent in each case.

Experiment number	107/08w plant number	Number of pollen grains	Number of seeds	Position of seeds
1	11	20	13	
2	14	50	12	
3	14	50	26	
4	14	60	12	
5	14	30	26	
6	25	60	10	
7	25	40	16	
8	25	40	11	
9	25	50	38	
10	25	15	2	
11	43	10	2	One seed in each cell; one at top and one in middle.
12	43	10	4	Four close together near top of one cell.
13	43	5	1	Middle.
14	43	2	0	
15	48	15	6	
16	48	18	2	
17	48	5	0	
18	48	8	2	
19	48	5	3	
20	48	14	7	Three in one cell, one at bottom and two in middle; four in the other cell, two at bottom and two in middle.
21	48	3	3	One at bottom of one cell; two in middle of the other cell.

A number of points of interest which might repay further study suggest themselves in connection with the results noted in the above table. The present data is too fragmentary to make possible any specific conclusions of general applicability. Such conclusions must wait upon a greater accumulation of evidence to allow of statistical treatment of the results. However, certain points brought out in the above table bear directly upon the question of the total sterility of the pollen of the F_1 *Tabacum-sylvestris* hybrids and upon the general abscission problem in *Nicotiana.*

It is evident that fertilization of an extremely small percentage of the ovules capable of fertilization[2] is sufficient to cause the flower to be held upon the plant rather than to be abscissed. We have for some time suspected that some such delicate balance existed between fertilization and the activation or formation of the separation layer. In

[2] Over two hundred viable seeds are produced by close pollination in *Nicotiana Langsdorffii* var. *grandiflora.*

this connection we may consider the three following possibilities in the case of the species under consideration. In the first place we may think of a ripe ovary containing at least two hundred ovules, each one properly matured and capable of the production of a viable seed following self-fertilization. After such fertilization and under normal conditions the seed capsule is retained on the plant up to and following the shedding of the ripe seed. On the other hand, another ripe ovary of the same plant in which no ovules are fertilized falls from the plant along with the other flower parts and a portion of the pedicel a few days after such maturity. Finally, in the case of a third ripened ovary on the same plant the fertilization of two or three ovules is sufficient to inhibit flower-fall. The fact that in experiments 14 and 17 in the above table but two and five ovules respectively could have been fertilized[3] is evidence in this connection. Additional evidence is found in the way in which the fruits of the F_1 *Tabacum-sylvestris* hybrids remain upon the plant when only a very few seeds are finally matured in them. In this case cytological examination of ripe ovaries showed that but a very small number of the six or seven hundred embryo-sacs are normal and capable of fertilization. When the viable pollen of either of the parent species is placed on the stigmas, the majority of the flowers thus pollinated remain attached to the plant and a few seeds are fully or partially matured in each ovary. We have here a case in which normally all the flowers on the plant fall as a result of lack of pollination with viable pollen and in which it is actually impossible for more than a very few ovules to be fertilized, yet when these few fertilizations are accomplished abscission does not take place.

Throughout the above discussion we have emphasized fertilization as contrasted with final formation of viable seed as the determining factor in the abscission of the flower. This has been done advisedly with the following facts in mind. In the F_1 *Tabacum-sylvestris* hybrids ripe seed capsules retained on the plant often contain nothing but a few empty seed cases. In other words it would seem that the fertilization of all or a number of the normally matured embryo-sacs provided the stimulus necessary to inhibit the activation of the separation layer, the question as to whether or not any of these fertilizations resulted in the formation of viable seeds being non-essential. Further, the results of experiments 14 and 17 in the above table indicate that

[3] The results in general indicate that branched pollen tubes with the necessary sexual elements do not occur in the style and thus that only one ovule is fertilized by a single pollen grain.

only the fertilization of an extremely small percentage of the normally matured ovules and possibly the completion of the earlier stages in embryo and endosperm development are necessary to make abscission impossible. The question of the inhibitory stimulus provided by the passage of pollen tubes down the style is still an open one. The fact that premature pollination in tobacco[4] has been shown to cause abscission lends support to the supposition that stylar penetration is important. In this connection the delicacy of the balance between stylar penetration or fertilization and abscission is again emphasized in that before the normal maturation of the female sexual elements pollination causes abscission, whereas at their complete maturity stylar penetration by a few pollen grains or fertilization of a few embryo-sacs is sufficient to inhibit such abscission.

In our experience there is in *Nicotiana* a certain stage of development of the seed capsule beyond which automatic abscission does not take place and spontaneous abscission cannot be induced. The explanation of this situation is found in the fact that mechanical tissue is rapidly developed in the pedicel of the flower somewhat after anthesis. A flower has only to pass the dangerous period when after cell separation (abscission) there is not sufficient mechanical tissue to hold it in position, to be retained permanently upon the plant. It is a question in such cases as experiments 14 and 17 above if abscission, induced by lack of normal seed development, may not occur after its normal time of occurrence. If it does so occur the unbroken cuticle and the more or less unaffected vascular bundle tissue both supported by the mechanical cylinder, may, on the one hand, prevent the drying out of the living but separated cells of the separation layer and, on the other, furnish the necessary supplies of water and metabolic ingredients to the developing seed capsule.

The results obtained in these experiments on controlled pollination appear finally to prove that no normal pollen is produced by the F_1 *Tabacum-sylvestris* hybrids. As noted above the flowers of such hybrids (with the exception of the parthenocarpic *Tabacum* "Cuba" hybrids) always fall when allowed to self-pollinate under bag. Many attempts extending over a number of years have been made to secure successful self-pollination artificially. A considerable excess of pollen has been applied in a single and in a number of successive pollinations, the stigma has been irritated with foreign substances prior to self-

[4] Hartley, C. P., Injurious effects of premature pollination, U. S. Dept. Agr., Bur. Pl. Ind., vol. 22, 1902.

pollination, artificial stigmatic fluids have been used, etc. In all cases the flowers have fallen. The results of microscopical examinations and pollen cultures have always indicated that no normal pollen was produced. Still it has always seemed possible that there might be a very few normal pollen grains corresponding to the percentage of normal ovules. That there is no such good pollen seems finally proved by the above results which show that two to five stylar penetrations or fertilizations are sufficient to inhibit flower-fall. If even one-half of one per cent of the thousands of pollen grains applied to the stigma were normally matured the flowers should have been retained upon the plant. Genetic incompatibility seemingly need not be considered in connection with this abscission evidence as to the pollen condition of these hybrids because it should not be expected to interfere with pollen tube growth and probably not with fertilization itself.

In the table data are given in a few instances as to the position on the placentae of the mature seeds. Thus some fragmentary evidence is given on the question of "selective" fertilization. Hartley (*loc. cit.*) states "That there is a close relation between the pollination of one half of the stigma and the setting of seeds in the corresponding half of the ovary is certain . . . ." No data are, however, given by Hartley as to the position of the seeds resulting in his experiments in which small amounts of pollen were used. Our results indicate that there is no selective fertilization from the point of view of position on the placentae and that the particular embryo-sacs reached by the pollen tubes is a matter of chance.

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IN
BOTANY

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AN APPARATUS FOR FLOWER MEASUREMENT

BY

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For a number of years we have been collecting data concerning the inheritance of flower size in hybrids between two varieties of *Nicotiana Langsdorffii.* It was early seen to be impracticable to attempt any measurements of fresh flowers in the field. For reasons discussed elsewhere[1] it seemed advisable to measure the first twenty-five flowers produced by each plant, and when from four hundred to eight hundred plants are involved this can be accomplished only by picking the flowers each day from every plant and preserving them in fluid (2% formalin) to be measured in the laboratory. For making these measurements the machine described below was devised. A description of it is given at this time because the results of our experiments cannot for the present be published, and it seems possible that others may be able to employ the apparatus, or some modification of it, in similar investigations. The original suggestion which led to the perfecting of the apparatus described was given to us by Dr. R. Goldschmidt, to whom our thanks are due. We are indebted to Mr. V. Arntzen of the Department of Civil Engineering of the University of California, who constructed the machine under our direction, for his helpful interest.

The accompanying drawing (pl. 54) is self-explanatory, and it is only necessary to describe the way in which the apparatus is used in making the flower measurements. The flowers of *N. Langsdorffii* are slender tubed, with a spreading limb provided with shallow and round-pointed lobes. The flowers are taken from the formalin solution, washed in water and the calyces removed. They are then cut with

[1] Goodspeed, T. H., and Clausen, R. E., Amer. Jour. Bot., vol. 2, pp. 332–374, 1915.

scissors at the point of union of limb and tube. This operation is simplified by the soft condition of the tissues after being immersed in formalin for some weeks, which causes the whole flower to straighten out when drawn from the washing water. The point at which the cut should be made is indicated by a slight groove occurring at the point of union of tube and limb. The limb and tube thus separated are placed in a dish of water so that the former may flatter out normally. It is then drawn up onto a piece of glass and transferred thus flattened to a large glass plate. The tube is then picked out of the water and placed beside the limb on the glass plate. This plate is cut approximately ten and one-fourth by twelve inches to fit into the frame of the measuring apparatus, where it is held firmly by the set screws *gg* (pl. 54).

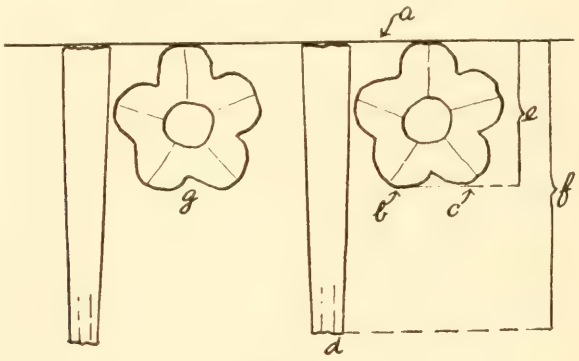
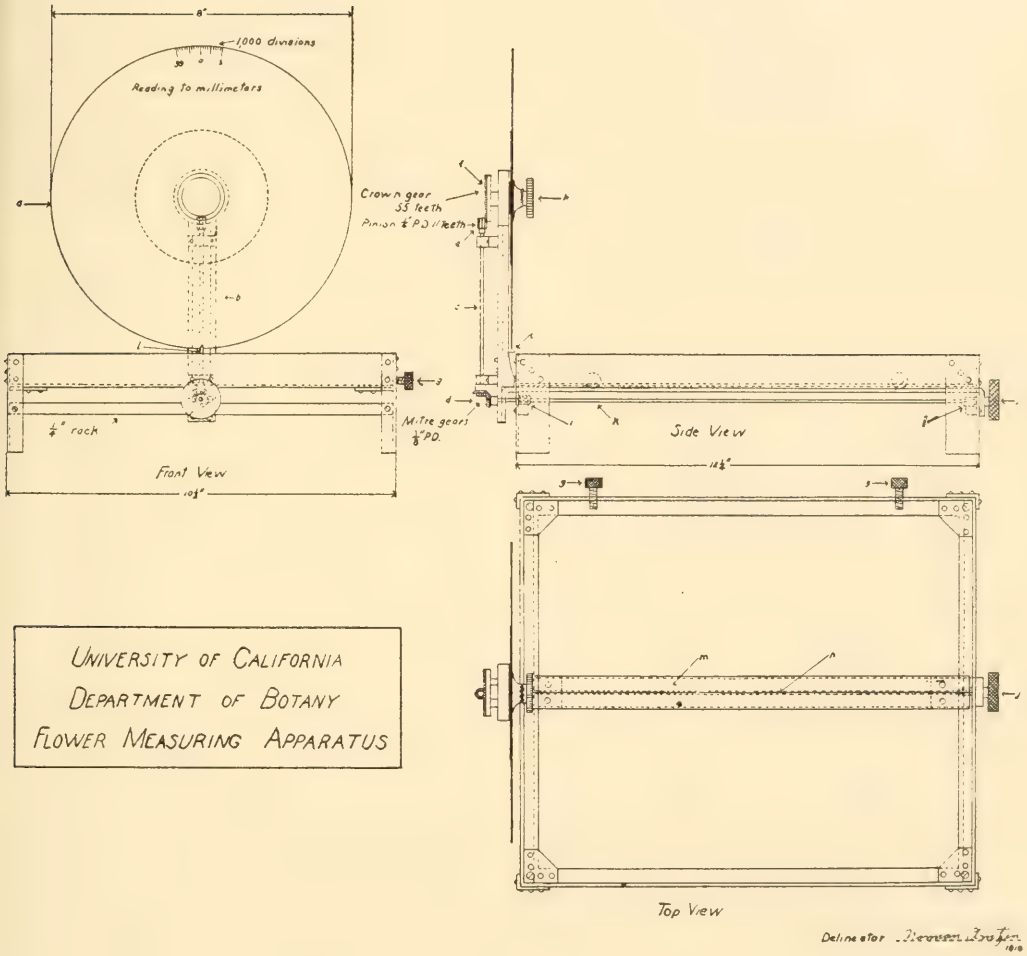


Figure 1

Parallel to the long axis of the glass plate two or three lines from two to three inches apart are drawn with a diamond. The cut ends of the tubes and one lobe of the limbs are brought to touch one of these lines. The arrangement is diagrammatically illustrated in figure 1. The size of the flowers will, obviously, determine the number which can be thus arranged on a single glass plate.

The flowers arranged as shown in figure 1 on the glass plate are put into the machine. The operator faces the circular disc (pl. 54, *a*) and the knurled head *j* (pl. 54) is turned to the right or left until the line *n*, marked on the movable bar *m*, is directly under the first of the lines scratched on the plate glass. By loosening the set screw *h* (pl. 54) the circular disc is swung around until the zero point is directly behind the indicator *l*. The set screw *h* is then tightened and the knurled head *j* is turned to the right until the line *n*, which is visible through the glass plate, is directly on a line with the corolla lobes *b*, *c* (fig. 1).



UNIVERSITY OF CALIFORNIA
DEPARTMENT OF BOTANY
FLOWER MEASURING APPARATUS

The diameter of the limb (fig. 1, *e*) is then read off in millimeters on the scale on the circular disc (pl. 54, *a*). The knurled head is now turned still more to the right until the line *n* is under the end of the tube (fig. 1, *d*). The length of the tube (fig. 1, *f*) is then read off. The bar *m* (pl. 54) is next turned back to the left to get the diameter of the second limb (fig. 1, *g*) and then moved to the right to obtain the length of the second tube and so on.

This apparatus or some modification of it would seem useful in measuring a wide variety of objects. Leaves, especially, can be measured on it very successfully.

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IN
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NOTE ON THE EFFECTS OF ILLUMINATING GAS AND ITS CONSTITUENTS IN CAUSING ABSCISSION OF FLOWERS IN *NICOTIANA* AND *CITRUS*

BY

T. H. GOODSPEED, J. M. McGEE AND R. W. HODGSON

I. INTRODUCTION

In our laboratories considerable attention has recently been given to the abscission problem. It came to the attention of one of us in connection with an investigation of the partially sterile F_1 hybrids between *Nicotiana Tabacum* varieties (cf. Setchell, 1912) and *N. sylvestris*.[1] A preliminary study of the mode of abscission in these hybrids was reported upon some time ago (Goodspeed and Kendall, 1916). As a continuation of this work, abscission of flowers and fruits in the *Solanaceae* and particularly in *Nicotiana* has recently been investigated (Kendall, 1918). Some studies have been made by one of us regarding the relation of the environmental complex to the occurrence of abscission and with reference to the mode of foliar abscission in *Citrus* (Hodgson, 1917, 1918). More recently attention has been called to the delicacy of the balance between fertilization and floral abscission (Goodspeed and Davidson, 1918). The present paper reports the results of experiments upon the effects of illuminating gas and its pure constituents in causing flower-fall, which represent a continuation of similar experiments by Kendall (*loc. cit.*) on *Nicotiana*, together with a report on experiments of the same type on the genus *Citrus*. The data submitted were considered preliminary at the time they were gathered. Their interest and the fact that other more important matters have put a stop indefinitely to any further accumulation of evidence make it seem desirable to present them at this time.

[1] Cf. various papers in Univ. Calif. Publ. Bot., vol. 5.

II. METHODS

The illuminating gas supplied to the laboratories of the University of California has the following composition by volume.

Nitrogen	4.10%
Oxygen	0.10
Hydrogen	55.00
Methane	26.30
Ethylene	5.00
Carbon dioxide	1.80
Carbon monoxide	7.70

In the experiments with *Nicotiana* the apparatus for subjecting the flowers to certain percentages of illuminating gas and its constituents consisted of five-litre bell-jars. In one case the bell-jar was fitted with a ground-glass stopper and stopcock and was set in a large crystallizing-dish so that the mouth of the bell-jar could be closed with a water seal. Part of the air in the jar was then drawn off and the jar partly filled with water, the volume of air was noted and the desired amount of gas being experimented with was introduced through the stopcock. In the other case a bell-jar was fitted with a ground-glass plate which sealed up the open end completely, the seal being made air-tight with vaseline. This bell-jar was fitted with two stopcocks and the gas being experimented with could thus be easily introduced and an equal volume of air simultaneously drawn off. In each of the experiments a portion of the terminal or lateral inflorescence upon which were seed capsules, open flowers and unopened buds was cut carefully from the plant and the cut end of the stem placed as quickly as possible in water in a 250 c.c. Erlenmeyer flask and the whole placed under the bell-jar. For each experiment a control was also kept in the open air. During the course of the experiments the flowers were shaken at frequent intervals to see if abscission had occurred. Flowering laterals from one plant of *Nicotiana Tabacum* var. *macrophylla purpurea* (U. C. B. G. 25/06) were used throughout.

In the experiments to determine the comparative effects of the constituents of illuminating gas upon floral abscission in this species of *Nicotiana* the gases were prepared as follows:

(*a*) Carbon dioxide—action of dilute c. p. sulfuric acid on pure sodium bicarbonate.

(*b*) Carbon monoxide—treating c. p. sodium formate with pure conc. sulfuric acid and washing by bubbling through strong sodium hydroxide solution.

(*c*) Hydrogen—electrolytically by electrolysis of distilled water containing sodium hydroxide, then passing through a tube containing soda lime and fused calcium chloride.

(*d*) Methane—treating sodium acetate with soda lime and washing by bubbling through strong sodium hydroxide solution.

(*e*) Ethylene—dropping ethyl alcohol (95%) into conc. c. p. phosphoric acid which was kept at a temperature of 210° to 220° C and washing by bubbling through cold conc. sulfuric acid and finally passing through a large U-tube filled with soda lime.

In the experiments with *Citrus* large dessicating dishes with ground-glass covers were used. In these containers flower-bearing shoots were placed and illuminating gas introduced. The reported percentages of gas used in this case (cf. table 1) are only approximations and were not quantitatively correct as in the corresponding experiments with *Nicotiana*. The controls were run in large moist chambers. *Citrus sinensis* varieties Washington navel and Valencia and *Citrus limonia* var. Eureka, growing in the greenhouses of the University of California, were employed in the experiments.

III. RESULTS

The results of the experiments to determine the effect of various percentages of illuminating gas in causing flower-fall in *Nicotiana* and *Citrus* are summarized in the following table.

TABLE 1

EFFECTS OF VARIOUS PERCENTAGES OF ILLUMINATING GAS IN CAUSING FLOWER-FALL IN *Nicotiana* AND *Citrus*

The figures given represent the times in hours between the start of the experiment and the fall of the first flower

Experiment number	Per cent illuminating gas	Nicotiana Tabacum var. macrophylla purpurea	Citrus sinensis var. Washington navel	C. sinensis var. Valencia	C. limonia var. Eureka
1	1.5	22			
	Control	44			
2	3.0		30	60	120
	Control		48	72	144
3	10.0	24			
4	10.0 (dry atmosphere)	48			
	Control	44			
5	20.0	23	42	72	144
	Control	43	72	96	150
6	50.0	22	42	50	130
	Control	42	70	96	140
7	75.0		40	66	150
	Control		66	100	180

The effects of the pure constituents of illuminating gas were investigated only in the case of *Nicotiana*. Table 2, which follows, summarizes the results of these experiments.

Table 2

Effects of the Pure Constituents of Illuminating Gas in Causing Flower-fall in *N. Tabacum* var. *macrophylla purpurea*

The figures given represent the times in hours between the start of the experiment and the fall of the first flower

Experiment number	Gas used	Per cent of gas	Time in hours
1	CO_2	10	17
	Control		34
2	CO	10	15
	Control		28
3	H_2	10	30
	Control		30
4	H_2	25	34
	Control		34
5	CH_4	10	48
	Control		48
6	C_2H_4	5	13
7	C_2H_4	5 (dry atmosphere)	14
	Control		20

In two experiments (table 1, experiment 4, and table 2, experiment 7) the material was subjected to the gases in a dry atmosphere. The bell-jar already described as fitted with a ground-glass plate sealed with vaseline was used in these experiments with a large amount of fused calcium chloride scattered over this ground-glass bottom. In all the other experiments with 25/06 water-sealed bell-jars were used.

In an additional experiment a lateral branch of 25/06 was exposed in an atmosphere containing 5% by volume of ethylene, but no abscission of the leaves could be induced, even after a considerable period had elapsed. In *Nicotiana Tabacum* there seems to be no direct leaf-fall in the sense that leaf bases are cleanly detached from the stem by the death or separation of cells. The dead, dry leaves remain attached to the plant often for months until the blades are broken away by the wind or other agencies. Thereafter the torn, dry leaf base may or may not come away cleanly from the stem. It seems apparent, however, that there is no abscission mechanism present in the leaf base, at least there is none which may be stimulated to activity by ethylene. Further investigation in this connection would appear to be profitable and necessary.

IV. DISCUSSION

Kendall (1918, p. 397) investigated the effects of 1.5 vol. % illuminating gas upon the abscission of flowers in fifteen species, varieties and hybrids of *Nicotiana* and in thirteen other genera and species of the *Solanaceae*. Flowering laterals were placed under the influence of the gas and air mixtures for fifteen hours, at the end of which time the amount and extent of abscission was noted. So far as *Nicotiana* is concerned he found that in four forms (cf. p. 398, table 6) neither buds, young flowers, flowers at anthesis, nor seed capsules were abscissed. In one case young buds only fell. In four cases all buds up to anthesis fell, but no older flowers nor seed capsules. In one form all flowers up to four or five days past anthesis were abscissed, and in three cases all buds and flowers fell. Finally, in one case it is recorded that buds, flowers and fruits were abscissed. Kendall also noted that when 3 vol. % of illuminating gas was used, or when the material was subjected to the 1.5 vol. % of gas for more than fifteen hours, some of the species previously found to be unaffected abscissed their flowers. His results in general indicated that there might be a more or less definite relation between the concentration of poisonous gases in the atmosphere surrounding the plant and the reaction time in spontaneous abscission.

With these results of Kendall's in mind, interest centered in subjecting the same material which he used to varying concentrations of illuminating gas and its constituents. The similar experiments upon induced flower-fall in *Citrus* varieties seemed of interest from a number of points of view. It has been frequently observed that in fumigating citrus trees with hydrocyanic acid in California there is a heavy leaf-fall, especially when certain weather conditions prevail. Apparently no observations have been made upon the effect of poisonous gases in causing flower-fall in this genus. The results here also serve as a check upon the data gathered from the similar experiments with *Nicotiana.*

From the results given in table 1, it is clear that the reaction time in spontaneous abscission is not appreciably hastened by increasing the percentage of illuminating gas surrounding the material under investigation. This fact is emphasized particularly in the case of *Nicotiana,* where the variations in reaction time both in the controls and in the material subjected to the gas are relatively slight. Miss Doubt (1917) has recently made a detailed study of the responses

of plants to illuminating gas. Flower-fall was not reported upon, but the response so far as leaf-fall is concerned received attention. A large number of species was placed in atmospheres containing various concentrations of illuminating gas and some of its constituents. A striking relation was found to exist between the concentration of the gas and the extent of leaf-fall. Thus, to take a few examples, in *Salvia splendens* 1000 p.p.m caused the oldest leaves at the base of the stem to fall, while 5 p.p.m. caused less shedding; in *Datura stramonium* 4000 p.p.m. caused the fall of all leaves except the youngest, whereas with 5000 p.p.m. only the oldest leaves fell, and in *Hibiscus rosa-sinensis* the same results were obtained when 4000 and 1000 p.p.m. were used. In this connection it is interesting to note that in the case of *Citrus* the opposite relation was found to hold, namely, that the youngest leaves were the first to be shed. Thus, in the case of a potted seedling subjected to an atmosphere containing illuminating gas, shedding began with the terminal leaves at thirty hours and gradually extended down the stem until all the leaves had fallen at fifty-four hours.

The distinction between flower and leaf abscission is emphasized in the relation, pointed out by Miss Doubt, between the age of the leaf and the readiness with which abscission is induced. She found that the older the plant, the less gas was required to cause the older leaves to fall; the younger leaves in all cases being least affected (cf. Lloyd, 1914, p. 69 and 1916, p. 57). This situation might be explained by the fact that in the case of the decidedly deciduous species the period during which the older leaves would normally be attached to the plant was less than that of the younger leaves and that thus the abscission layer was already partially formed or more readily activated. In the absence of controls further speculation on this point is useless. In contrast to these responses of younger and older leaves, we have the following situation in the case of floral abscission, at least so far as *Nicotiana* is concerned. Here the period preceding the partial maturing of the fruit is, almost without exception, the only one in which flower-fall can take place, since the rapid development of mechanical tissue makes fall nearly impossible thereafter, even if a separation layer is found among the cortical cells which remain undifferentiated. Thus buds, young flowers, and partially matured seed capsules alone are shed—and the latter only rarely. To take one example, it is a common experience in the field to find that as a result of low temperature there is a progressive fall of the smallest buds and then larger

ones, while open flowers just before anthesis and older flowers are apparently not at all affected.

Miss Doubt mentions the fact that some species would give no visible response during or immediately following the period of exposure to illuminating gas, but that a number of days after the end of the treatment more or less heavy leaf-fall would occur. This is an interesting point, especially when one considers its implications with regard to the factors actually responsible for initiating cell separation. Further, a specific effect of different gases which bring about spontaneous abscission is suggested in view of the fact that, as contrasted with Miss Doubt's results with illuminating gas, Moore and Willaman (1917) found that fumigation with hydrocyanic acid causes an increase in permeability of the leaf septa, which is followed by a wilting, etc., from which, however, the treated plants after a time appear to recover completely. It may be noted in this general connection that Hannig[2] (Lloyd, 1914, p. 68) states that 0.00002 vol. % of illuminating gas caused flower-fall in *Mirabilis*, etc., while high concentrations employed for fourteen hours did not give the same result directly but did give it indirectly somewhat after the end of the treatment. Lloyd (1916, p. 58) states that cotton bolls "may be shed when one or two days old in relatively high frequences, in response to stimuli applied before anthesis, provided the stimuli applied are severe enough."

In the case of the experiments with *Citrus* the controls show a rather wide variation which is sufficient to account for the variation in reaction time shown by the material subjected to gas. It will also be remembered that the percentages of gas stated in table 1 were not so quantitatively correct as in the similar experiments with *Nicotiana*. Except in the case of variety Washington navel the effect of illuminating gas is very slight and for *C. limonia* one might almost be led to suspect from the results given that gas has no effect in accelerating reaction time. It seems possible that with the exception of variety Washington navel there is a type of rigor effect in the case of the higher percentages of illuminating gas which inhibits all normal response. Fitting (1911), who showed that laboratory air, carbon dioxide, tobacco smoke, ether, etc., cause premature fall of corolla, also found that rigor induced by heat effects and lack of oxygen tend to inhibit the injurious effects of poisonous gases. Of interest in connection with this whole question of the influence of poisonous and anesthetizing gases is the work of Hempel (1911), who has shown that

[2] We have not had access to the original of Hannig's article.

ether effects are dependent upon its concentration. Normal destruction of proteins in the germination of *Pisum* and *Lupinus* was retarded by doses up to approximately 0.01% by volume, but the process was accelerated in strong doses. Also Johannsen (1896) found that certain concentrations of ether and chloroform caused an increase in soluble sugars and in the decomposition of proteins in bulbs of *Crocus* and seeds of barley and pea, while very weak doses gave the reverse effects, i.e., favored starch and protein synthesis.

The definite response of variety Washington navel as compared with the other *Citrus* material is quite in keeping with the normal reaction of these plants whereby under certain conditions of temperature and humidity they drop their immature fruits, while corresponding environmental conditions have a much less corresponding effect upon other species of *Citrus*. In this connection attention must be called to the differences in the average reaction times for the controls, in that flowers of variety Washington navel fall in respectively two-thirds and one-half the time that is necessary for flower-fall in the other two forms. This gradient is rather remarkable from a number of points of view and our results should be confirmed and extended and similar determinations made for other *Citrus* varieties.

The experiments in which the pure constituents of illuminating gas were employed were marred by our inability to maintain constant temperatures during their course. The influence of variations in atmospheric temperature in such abscission experiments was apparent from the start and some preliminary efforts were made to determine it. One of a series of the moist chambers, noted above as employed for the controls in the experiments with *Citrus*, was placed in an incubator and run for some time at approximately ten degrees above room temperature. Foliar abscission under such conditions appeared at the end of thirty hours, while the control run at room temperature showed the first leaf-fall at fifty-six hours. The variation in the reaction time of the controls given in table 2 is rather great, but the results of experiments 3, 4, and 5 in this table show that this variation does not need to be given too much weight in connection with the effects of the various gases in causing abscission. This variation, however, appears to be indicative of changes and instability in the general physiological condition of the plants under investigation. The experiments listed in table 2 were carried on at practically the end of the normal growing period of the plants used and ordinary experience and the results of other workers would suggest that this fact might influence to some extent reactions to specific stimuli (cf. Lloyd, *loc. cit.*, p. 69).

In experiments 3, 4, and 5 (table 2) the material was placed in contact with 10 vol. % and 25 vol. % of hydrogen and 10 vol. % of methane, respectively. The time required for the first flower to fall was the same in the treated material as in the controls. The results of the experiments in which hydrogen was employed are included primarily to emphasize the point mentioned in the paragraph above, as there is no reason to suppose that this gas would be effective in initiating abscission.

The result of experiment 5, in which 10 vol. % of methane was used, shows that this gas has nothing to do with the flower-fall caused by illuminating gas. Knight and Crocker (1913) in studies of toxicity of tobacco smoke found that methane in 0.00001% concentration was not toxic to the etiolated hypocotyl of the sweet pea, carbon dioxide when washed out of the smoke in no wise decreased its toxicity, and that the toxicity of ethylene is so extreme that 1 part in 10,000,000 has a marked effect.

As will be noted in table 2, relatively high concentrations of carbon dioxide, carbon monoxide, and ethylene are effective in bringing about flower-fall in decidedly less than the normal time. The effect of ethylene was to have been anticipated in view of the evidence furnished by a number of investigations which indicate that this gas not only is able to produce marked physiological changes in the plant, but also that to its presence in illuminating gas is attributable the responses which plants give when subjected to small amounts of that substance The results of Knight and Crocker already referred to emphasize this point. Harvey (1913) found for the seedling castor oil bean that the cotyledons fell or the abscission layer was formed after exposing the plants to 1 p.p.m. of ethylene or to 25 p.p.m. of illuminating gas (in this case equal to 1 p.p.m. of ethylene). Doubt (*loc. cit.*) showed that in various species 5 to 8 p.p.m. of ethylene caused leaf-fall. In at least two cases it is worthy of note that no leaf-fall was reported after treatment with 8 p.p.m. of ethylene, although fall occurred when 4000 p.p.m. illuminating gas was used. Undoubtedly fall would have occurred if the concentration of ethylene had more nearly approached that in which it was present in the concentration of illuminating gas which did cause fall.

Our experiments upon the effectiveness of the constituents of illuminating gas in causing flower-fall are of value mainly in so far as they indicate that carbon monoxide and carbon dioxide are effective. As mentioned above Knight and Crocker (*loc. cit.*) found that carbon

dioxide was not a toxic constituent of tobacco smoke and Miss Doubt (*loc. cit.*) states that carbon monoxide had no effect in bringing about leaf-fall. Carbon dioxide, in Fitting's (1911) experiments, caused shedding of the corolla prematurely. Brown and Escombe (1902) found for a number of species that in 11.4 parts in 10,000 inflorescence was almost totally inhibited, while in *Nicotiana affinis* and *N. sylvestris* the small flower buds which began to form were all shed long before anthesis. Hannig (1913) found that carbon dioxide was not effective in causing premature floral abscission.

As will be noted in the case of experiments 3 and 4 in table 1, the flowers fell at the end of forty-eight hours in a dry atmosphere containing 10 vol. % of illuminating gas and after approximately the same length of time in the case of the control, whereas in a moist atmosphere containing the same per cent of illuminating gas the flowers fell in twenty-four hours. This situation emphasizes the fact that there is a definite moisture requirement for the abscission process. It has been apparent throughout our field experience with *Nicotiana* that severe floral abscission often follows sudden changes in temperature, especially when such changes are accompanied by rain or great atmospheric humidity. The rôle and significance of turgor in the abscission process has been fully dealt with elsewhere (cf. Goodspeed and Kendall, 1916 and Kendall, 1918). The conditions obtaining in experiments 6 and 7 in table 2 were the same as in the experiments last referred to, except that 5 vol. % ethylene in place of 10 vol. % illuminating gas was employed. Here, however, the reaction time in the dry and the moist atmospheres was approximately the same, while the first flower in the control fell after a considerably longer period. This may simply be another illustration of the extreme toxicity of ethylene or may possibly be due to the fact that this experiment was performed late in the normal growing season of the plant, with a consequent greater instability of many of the vital physiological processes.

Our data are in general too fragmentary to permit the drawing of any conclusions as to the basic cause of abscissional responses to poisonous gases. It seems probable that these phenomena will finally be proved to be due to modifications in the normal water relations of the plants concerned. There is abundant evidence that such modifications caused by natural or artificial reduction of the absorbing surfaces or by increased transpiration under conditions of high temperature and low humidity will bring about abscission. Poisonous gases

may be thought of as bringing about disturbances in normal water relations by increasing the permeability of the leaf septa to favor abnormally high water loss or by a paralysis of the stomatal apparatus with corresponding results. On the other hand, it is at least conceivable that a shock effect resulting in a rather complete disturbance of physiological equilibria may be the basic cause of the observed abscission response of plants subjected to poisonous gases.

V. SUMMARY

The experiments reported upon in detail above were performed to give evidence as to the effect of various concentrations of illuminating gas and of its constituents upon the abscission of flowers of *Nicotiana Tabacum* var. *macrophylla purpurea, Citrus sinensis* varieties Washington navel and Valencia and *C. limonia* var. Eureka.

1. In *N. Tab.* var. *macrophylla purpurea* the presence of illuminating gas in the atmosphere surrounding flowering laterals caused the first flowers to fall in approximately one-half the normal time. The reaction time was approximately the same in all concentrations of illuminating gas.

2. Of the constituents of illuminating gas, carbon monoxide, carbon dioxide and ethylene caused premature abscission.

3. All the experiments with the exception of two were carried on in a moist atmosphere. In these two cases the effect of a dry atmosphere containing in the first case 10 vol. % illuminating gas and in the second 5 vol. % ethylene was observed. In the first, abscission was so retarded that the first flower fell after approximately the same length of time as the control. In the second, the first flower fell almost as soon in the dry as in the moist atmosphere, and in both cases considerably earlier than in the control.

4. In the case of *C. sinensis*, variety Washington navel exhibited a marked abscissional response to illuminating gas and did so irrespective of its concentration. In illuminating gas *C. sinensis* variety Valencia and *C. limonia* variety Eureka showed little or no increase in the reaction time of the abscission process.

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NOTES ON THE GERMINATION OF TOBACCO SEED III

NOTE ON THE RELATION OF LIGHT AND DARKNESS TO GERMINATION

BY
T. HARPER GOODSPEED

The recent paper of Honing[1] reopens the question of the light requirements for germination in *Nicotiana*. For a number of years the writer has been convinced that the seed of the great majority of species of *Nicotiana* would germinate in darkness and that the numerous statements to the contrary were based upon experiments not accurately controlled. An examination of the voluminous literature dealing with the influence of varying amounts of illumination upon the germination of seeds leaves no doubt that temperature and moisture effects are contributing factors in the results obtained, and that the stage of maturity of the seed employed is a matter to be carefully determined. Finally, when one considers that from the strictly physiological point of view the influence of light upon germination corresponds to that of light upon growth phenomena in general as an initiating stimulus and a source of energy, the need for the most exact methods of experimentation is indicated.

The following results dealing with the influence of light and darkness upon the germination of five varieties of *Nicotiana Tabacum* and five of *N. rustica* were obtained from experiments which are looked upon as preliminary to a more thorough and better controlled investigation. Since the results listed are so definite and since the larger investigation contemplated must wait upon the completion of more important matters, it seems well to present briefly the data at hand.

[1] Honing, J. A., The influence of light on the germination of the seeds of different varieties of *Nicotiana Tabacum*, Bull. Deli Proefstat., December, 1916.

APR 1 1919

A thorough review of and commentary upon the literature dealing with the light requirements for germination in general appeals to the writer as important in view of the divergent results obtained by different investigators. Such a review is in progress and in the present report no attempt will be made to correlate the results listed with those of other workers.

I am indebted to Miss E. E. Keith for valuable assistance in setting up the experiments and recording the germinations.

The following list of varieties of *N. Tabacum* and *N. rustica* with their numbers in the University of California Botanical Garden (U. C. B. G.)[2] will serve to identify the individuals included in the table and discussion below.

12/07—*Nicotiana rustica* var. *asiatica.*
13/07—*N. rustica* var. *brasilia.*
14/07—*N. rustica* var. *humilis.*
15/07—*N. rustica* var. *jamaicensis.*
16/07—*N. rustica* var. *scabra.*
22/07—*N. Tabacum* var. *macrophylla.*
25/06—*N. Tabacum* var. *macrophylla purpurea.*
71/05—*N. Tabacum* "Brazilian."
72/05—*N. Tabacum* "Cavala."
78/05—*N. Tabacum* "Maryland."

The seed was prepared for germination as described in previous papers.[3] For certain of the experiments (cf. table, note 2) a glass-sided germinating case was provided. In this germinating case a 40-watt tungsten electric light bulb was hung and the ventilation so regulated that the temperature was approximately constant at 30°C. The seeds to be subjected to continuous darkness were placed in paper collar-boxes and the boxes put into the germinating case. The duplicates were placed in the germinating case exposed to the light of the electric bulb day and night. In the remaining experiments no attempt was made to regulate the temperature, and the seeds in collar-boxes and undarkened were placed near a window but at no time in direct sunlight. For any two sets of the same seed—one in darkness and one in diffuse light—the range of temperature variation was the same when the germination took place out of the germinating case, as in all such cases the two tests were set up on the same day. The tests were examined on the days indicated in the table below and the

[2] Cf. Setchell, W. A., Studies in Nicotiana I, Univ. Calif. Publ. Bot., vol. 5. no. 1, pp. 1–86, 1912.

[3] Goodspeed, T. H., Notes on the germination of tobacco seed, Univ. Calif. Publ. Bot., vol. 5, no. 5, pp. 199–222, 1913; *idem, ibid.*, vol. 5, no. 7, pp. 233–248, 1915.

germinated seeds picked out. The criterion of germination was the same as that noted in a previous paper.

The breeding experiments with *Nicotiana* conducted in the University of California Botanical Garden cover a considerable number of years; thus we will be able to investigate the light requirements for germination in the case of seed of various ages. Attention might again be called to the fact that the seed used in our experiments is pedigreed seed representing in each case the product of individual plants of species and varieties of *Nicotiana* grown in the pure line. The writer's previous reports have indicated the remarkable viability of old tobacco seed. In the table below it will be noted that in the case of the *N. Tabacum* varieties seed varying in age from nine to twelve

Test number	Year	Plant designation	Number of seeds that germinated on the days indicated															Percent germinated	Age of seed in years	Relative germination[3]	Day of maximum germination
			7	8	9	10	11	12	13	14	15	16	17	18	19	20	20+				
1	1916	12/07	80	..	..	9	....	..	..	..	....	..	....	....	....	....	....	89	2	3	7
2	1916	12/07	**63**[1]	..	..	**19**	....	..	..	..	**3**	....	....	....	**5**	....	....	**90**	2	.	**7**
3	1916	13/07	50	..	..	30	....	....	..	..	4	..	..	....	....	....	5	89	2	4	**7**
4	1916	13/07	**54**	..	..	**24**	....	..	..	..	**1**	..	....	..	..	....	**3**	**82**	2	.	**7**
5	1916	14/07	88	....	..	5	....	....	....	....	....	....	....	....	....	....	....	93	2	5	**7**
6	1916	14/07	**21**	....	..	**30**	....	....	....	....	....	....	....	....	....	....	....	**51**	2	—	10
7	1916	15/07	94	....	..	....	....	....	....	....	....	....	....	....	....	....	....	94	2	....	**7**
8	1916	15/07	**95**	....	....	....	....	....	....	....	....	....	....	....	....	....	....	**95**	2	+	**7**
9	1916	16/07	48[2]	....	..	....	....	..	36	....	....	....	....	....	....	....	8	92	2	....	**7**
10	1916	16/07	**73**	....	..	....	....	....	..	....	....	....	....	....	....	....	**21**	**94**	2	+	**7**
11	1909	22/07	....	....	....	66	....	....	9	....	....	....	....	....	....	....	....	75	9	....	10
12	1909	22/07	....	..	..	....	..	....	**67**	....	....	**13**	....	....	....	..	**6**	**86**	9	+	13
13	1916	22/07	80	....	....	....	....	....	7	....	....	....	....	....	....	....	....	87	2	....	**7**
14	1916	22/07	**73**	....	..	....	..	....	....	....	....	....	....	....	....	....	....	**73**	2	—	7
15	1906	25/06	..	....	....	..	....	19	10	3	....	6	..	3	4	..	....	45	12	..	12
16	1906	25/06	..	....	....	..	....	**22**	**11**	**15**	....	**6**	....	**7**	**4**	..	....	**65**	12	+	12
17	1907	25/06	..	....	....	..	....	21	24	22	....	4	1	..	1	..	....	73	11	....	13
18	1907	25/06	..	....	....	..	....	**34**	**17**	**11**	..	**11**	..	**2**	....	..	....	**75**	11	+	12
19	1909	25/06	....	85	5	..	..	....	....	....	....	....	....	....	....	..	....	90	9	....	8
20	1909	25/06	....	**59**	....	....	....	**3**	....	....	....	....	**6**	....	....	....	....	**68**	9	—	8
21	1911	25/06	....	....	....	....	....	....	....	....	17	38	1	....	24	....	....	80	7	....	16
22	1911	25/06	..	....	..	....	....	....	**1**	**3**	....	**5**	**40**	....	**25**	....	....	**74**	7	=	17
23	1912	25/06	..	..	....	....	43	25	..	2	....	11	....	....	..	..	....	81	6	....	11
24	1912	25/06	..	..	....	..	**46**	**8**	**13**	**6**	....	**5**	....	....	**2**	..	....	**80**	6	=	11
25	1916	25/06	..	92	..	2	....	....	....	....	....	....	..	....	....	..	....	94	2	..	8
26	1916	25/06	..	**78**	..	**5**	....	....	....	....	....	....	....	....	..	....	....	**83**	2	.	8
27	1909	71/05	....	52	38	9	....	....	....	....	....	....	....	....	....	....	....	99	9	....	8
28	1909	71/05	....	..	..	..	**66**	**14**	..	..	**1**	....	....	..	..	....	..	**81**	9	=	11
29	1916	71/05	....	84	..	5	2	....	....	....	....	....	....	..	..	....	..	91	2	..	8
30	1916	71/05	....	**82**	....	**13**	....	....	....	....	....	....	....	....	....	....	....	**95**	2	-	8
31	1909	72/05	....	....	..	....	..	80	....	4	1	..	....	..	....	..	....	85	9	..	12
32	1909	72/05	....	....	..	....	..	**60**	....	**16**	**1**	..	....	**1**	..	..	....	**78**	9	=	12
33	1916	72/05	..	86	.	....	....	2	....	....	....	....	....	....	..	..	....	88	2	....	8
34	1916	72/05	....	**55**	....	**29**	....	..	....	**6**	....	....	....	....	..	..	....	**90**	2	+	8
35	1909	78/05	.	.	.	.	.	..	....	5	....	55	....	..	..	..	25	85	9	.	16
36	1909	78/05	....	.	....	.	.	....	....	....	....	....	....	....	....	..	**57**	**57**	9	—	20+
37	1916	78/05	....	.	....	10	.	8	10	.	....	7	....	..	5	..	9	49	2	.	13
38	1916	78/05	.	.	....	**8**	.	**20**	**10**	**13**	....	.	....	..	**3**	....	**7**	**61**	2	.	12

[1] The figures in bold face type refer to seed germinated in continuous darkness.

[2] Up to this point the figures in ordinary type refer to seed germination in continuous light, the source of light being a 40-watt tungsten electric light bulb. The remaining figures in ordinary type refer to seed germinated under diurnal illumination.

[3] "+" indicates that the per cent of seeds germinated in darkness exceeded the per cent germinated under continuous or diurnal illumination.

[4] "=" indicates that germination under the two conditions was the same, a difference of less than ten seeds being the criterion.

[5] "—" indicates that germination in darkness was decidedly less than under continuous or diurnal illumination.

years was available. In the majority of cases the germination in light and in darkness of nine year old as compared with two year old seed was tested. For 25/06 (tests 15 to 26) the germination under the two conditions of twelve, eleven, nine, seven, six, and two year old seed was investigated. Two year old seed only of five *N. rustica* varieties was employed. As will be seen in the table the older seed germinated with a good or fair percentage, although the germination is scattering and the period of maximum germination is later than in the case of younger seed of the same pedigree.

Various points are brought out in the table above which deserve further investigation. Thus, for example, the poor germination of two year old seed (tests 37 and 38) as compared with nine year old seed (tests 35 and 36) of 78/05 is unusual and may indicate a lessened viability due to continued inbreeding. This matter is especially interesting in view of the fact that two year old seed of 78/05 germinated much more heavily in darkness than in light. Again, the heavy germination of the nine year old seed of 71/05 (tests 27 and 29) is worthy of notice.

The notation used in the seventh column of the table to indicate the relation between the germination of seed of the same year in light and in darkness is merely one of convenience and has no particular significance. Thus the "+" sign is employed simply to emphasize the fact that the per cent of germination of the seed in darkness was actually greater than that of the corresponding seed subjected to continuous illumination or under diurnal illumination. In reality practically all those pairs of tests marked "+" fall into the "=" section since germination in darkness rarely exceeded germination in light by more than ten seeds.

There is no doubt that the seed of five representative types of *N. Tabacum* and of five varieties of *N. rustica* will germinate readily in darkness. These five varieties of *N. Tabacum* represent a large proportion of the basic types from which the commercial strains of American tobacco, with which Honing worked, have been derived. Honing (*loc. cit.*, p. 14) found that of twenty-one strains of *N. Tabacum* received by him under trade names from the United States Department of Agriculture and germinated in darkness more than fifteen germinated only 20 per cent or less and that none of the twenty-one germinated over 50 per cent. I am at a loss to explain this result of Honing or a number of the others which he reports. Since the germinations listed in the above table indicate that germina-

tion in darkness may be slow and scattering, Honing's conclusions might have been different had he followed the progress of his germinations in darkness over a longer period.

In addition to the general conclusion that *N. rustica* and *N. Tabacum* varieties will germinate in darkness, another point of interest is suggested by the results given in the above table. Thus it will be noted that old and new seed will germinate equally well in darkness. Of nine tests of seed from six to twelve years old only two showed a smaller per cent of germination in darkness than in light. Similarly of ten tests of two year old seed, eight are marked "+" or "=." A confirmation of this apparent phenomenon and any attempt to comment upon its significance must wait upon a more detailed investigation.

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INHERITANCE IN NICOTIANA TABACUM

I

A REPORT ON THE RESULTS OF CROSSING CERTAIN VARIETIES

BY

WILLIAM ALBERT SETCHELL, THOMAS HARPER GOODSPEED, AND ROY ELWOOD CLAUSEN

CONTENTS

PAGE

I. Introductory ... 458
II. Plan of the work ... 460
III. *Angustifolia-macrophylla* series ... 462
1. Parents of the *angustifolia-macrophylla* series ... 463
2. F_1 of the *angustifolia-macrophylla* series ... 467
3. F_2 of the *angustifolia-macrophylla* series ... 469
4. F_3 and subsequent generations of the *angustifolia-macrophylla* series ... 472
a. Stenophylla derivatives ... 475
b. Latifolia derivatives ... 476
c. Lanceolata derivatives ... 478
d. Loriifolia derivatives ... 478
e. Auriculata derivatives ... 479
f. Sessilifolia derivatives ... 480
5. Summary of flower color observations in F_2 and subsequent generations 482
6. Later sowings of F_2 and F_3 of the *angustifolia-macrophylla* series ... 483
7. Crosses of derivatives with the parents ... 487
8. Discussion of results of the *angustifolia-macrophylla* series ... 490
IV. *Calycina-virginica* series ... 494
1. Parents of the *calycina-virginica* series ... 494
2. F_1 of the *calycina-virginica* series ... 496
3. F_2 of the *calycina-virginica* series ... 497
4. F_3 and subsequent generations of the *calycina-virginica* series ... 499
5. Discussion of results of the *calycina-virginica* series ... 504
V. *Alba-macrophylla* series ... 504
1. Parents of the *alba-macrophylla* series ... 504
2. F_1 of the *alba-macrophylla* series ... 505
3. F_2 of the *alba-macrophylla* series ... 505
4. F_3 and subsequent generations of the *alba-macrophylla* series ... 507
5. Discussion of results of the *alba-macrophylla* series ... 510

PAGE
VI. General Conclusions 510
1. Origin and interrelationships of varieties of *Tabacum* 510
2. Methodology of Mendelian analysis in *Tabacum* 513
3. Mendelian heredity in *Tabacum* 516
VII. Summary 520
Literature cited 520
Explanation of plates 522

I. INTRODUCTORY

The inception of the work on the various species of *Nicotiana* grown and bred in the University of California Botanical Garden has already been sketched in a previous number of this series (cf. Setchell, 1912). As stated there, the original intention was to assemble a collection of tobacco plants simply as a portion of the outfit of the Botanical Garden for general instruction and display. So great was the variety and evident misapplication of the names under which the seeds were received, however, that it seemed advisable to attempt to determine, as definitely as possible, the status of each plant.

In this connection, the work of Comes, in particular, came under consideration and especially his views as to the origin and interrelationships of the various cultivated forms belonging to the *Tabacum* group. Comes (1899, p. 4 and elsewhere) regards the numerous cultivated forms of tobacco as having originated in various ways from certain fundamental varieties. He estimated that there are six of these fundamental varieties of *Tabacum*, and he supposed the large number of various and seemingly more or less intergrading forms to have arisen through the influence of the forces of acclimatization, adaptation, hybridization, and selection. Of these, undoubtedly, the greater variations have been produced and perpetuated, according to the ideas of Comes, through hybridization and selection. In his monograph (1899) and in his later more exhaustive treatise (1905), Comes has attempted to estimate just which of his six fundamental varieties of *Tabacum* have coöperated in producing each one of the cultivated "races" so far as known to him.

The statements of Comes as regards the constitution of his various races seem to have been based on the results of morphological study rather than upon breeding analysis. The advisability occurred to the senior author of attempting to test Comes' hypothesis by selecting varieties seemingly fundamental in type, and through hybridization

and selection attempting to secure constant races exhibiting various recombinations of the parental characters. The work thus conceived has been carried out in detail in a certain few but seemingly characteristic cases. Several different crosses were made in 1909, the first filial generations were grown in 1910, and each year since that time has seen successive filial generations in the field.

Although the *Nicotiana* investigations were originally designed to attack experimentally a comparatively simple and definite problem, they have since been greatly amplified in scope. At the present time three rather distinct lines of investigation are actively in progress, viz.,

1. Mendelian inheritance in *N. Tabacum.*
2. Inheritance of quantitative characters.
3. Studies of interspecific hybrids.

The recent appearance of bud variations in hybrid lines favorable for an analytic study of that phenomenon has resulted in the addition of another research project. Now it has been found that, although seemingly distinct, progress in these separate lines of investigation is more or less interdependent. In particular it has been found that certain of the phenomena exhibited in interspecific hybrid populations from crosses between *N. sylvestris* and varieties of *N. Tabacum* require for satisfactory analysis and explanation an accurate and detailed knowledge of the Mendelian differences which exist among the particular varieties of *N. Tabacum* that have been used in those investigations. Accordingly in later years these studies of hybrids between varieties of *N. Tabacum,* originally designed merely to test experimentally the interrelationships existing among such varieties, have been directed toward a specific Mendelian analysis of the germinal differences existing in a selected set of varieties.

With this change in emphasis has come a full appreciation of the difficulties of Mendelian studies in *N. Tabacum.* It has been very evident that, for the most part, the character differences among varieties of *N. Tabacum* do not rest upon a simple genetic basis; on the contrary, they often depend upon very complex and involved Mendelian differences; so that in segregating populations it is often impossible to demonstrate the existence of definite, discontinuous character classes. Not uncommonly the members of such populations may be arranged in series connecting by imperceptible differences the most extreme character expressions in the population.

But although complex intergrading segregation has often been observed in F_2, it has not been found that such complex segregation

persists in subsequent generations in the hybrid lines. On the contrary, it has been found, as will become evident in a study of the experimental material communicated herewith, that a great simplification occurs in the segregation in F_3 and subsequent generations, and that continuous segregation gives way to discontinuous just as might be expected from Mendelian theory. By observing the segregation in the consecutive generations of hybrid lines which have become homozygous in most of their loci through self-fertilization, it is possible to obtain some idea of the Mendelian factor pairs involved in the character contrasts and of their relations to one another. It has also proved possible by a few years of self-fertilization to establish stable lines representing recombinations of parental characters. By investigating the interrelations among such stable derivative lines, which obviously should differ in fewer factors from one another and from the original parental varieties than the parental varieties differ from each other, it would seem possible to develop an indirect mode of attack by which the Mendelian analysis could be refined to any desired extent. The original plan of the investigation, therefore, having as its purpose a demonstration of the possibility of securing by hybridization stable derivative lines representing recombinatons of characters contained in the parents and comparable to the numerous existing varieties of *N. Tabacum,* has been diverted into a detailed study of Mendelian differences among a typical set of *N. Tabacum* varieties.

II. PLAN OF THE WORK

In the introductory paper the senior author has discussed the fundamental types of *N. Tabacum*, and as indicated there, has expressed a preference for selecting some five fundamental varieties, or species, as representing the basal morphological elements found, or seemingly to be detected, in cultivated races of *N. Tabacum.* There is no necessity for discussing further, at present, the reasons for preferring the particular types selected by us as against those of either Comes or Anastasia (1906), since the fundamental conceptions agree sufficiently well and the important thing has been to make a beginning in experimentation by using varieties which present seemingly fundamentally different character complexes in most characteristic form in plants breeding true to type in the pure line. Certain reasons for selecting a particular type or types will be discussed in connection with the

consideration of the various crosses. Besides the "fundamental" types, there have been selected for crossing certain other types, possibly fundamental, or in some cases derivative, which have been employed for testing the inheritance of some particular character or group of characters. All of these have been described in the first paper of this volume.

The taxonomic problems in *N. Tabacum* do not appear to differ from those presented by many other species of cultivated plants. Barley, maize, oats, rice, wheat, among others, exhibit a similar diversity of forms with more or less obvious class distinctions. In these as in *N. Tabacum* it appears to be an easy task to shuffle and recombine characters indefinitely. Clearly there can be no segregation of forms into distinct species on genetic grounds; the basis of speciation, if any, must depend either upon convenience merely or what amounts to practically the same thing, upon elevation of certain Mendelian character contrasts to a higher rank in classification than others. Since the taxonomic problem, therefore, is not strictly a genetic one, it seems best to follow general usage in this respect, referring all the polymorphic assemblage of forms to the one species *N. Tabacum*, and regarding the several races included thereunder as varieties of equal rank.

The varieties employed in this series of investigations are: *N. Tabacum* var. *alba*, U. C. B. G. 30/06, previously described by Setchell as "White" Tobacco; *N. Tabacum* var. *angustifolia*, U. C. B. G. 68/07, previously described by Setchell as *N. angustifolia; N. Tabacum* var. *calycina*, U. C. B. G. 110/05; *N. Tabacum* var. *macrophylla*, U. C. B. G. 22/07; and *N. Tabacum* var. *virginica*, U. C. B. G. 78/05, previously described by Setchell as *N. Tabacum* "Maryland." In each instance the University of California Botanical Garden (U. C. B. G.) number contains in the numerator the accession number of the year given in the denominator. The varieties have in the majority of cases been grown in pure lines from the date of their receipt. In order to avoid needlessly encumbering the text with scientific names, the varieties mentioned above will be referred to by their varietal designations only, and when reference is made to the whole group the species name *Tabacum* will be used alone.

Three series of cultures are described in the present article: the *angustifolia-macrophylla* series, which has been derived from reciprocal crosses of *angustifolia* and *macrophylla;* the *calycina-virginica* series, derived in the same way from *calycina* and *virginica;* and the *alba-*

macrophylla series, from *alba* and *macrophylla*. In the course of the investigations other crosses were made between different varieties of *Tabacum* and to a limited extent between other species of *Nicotiana;* but the principal attention has been paid to the three crosses noted above, and they and their progenies alone will be considered in the present paper. It may be said at this point that the different varieties of *Tabacum* cross readily with one another, giving an abundance of good viable seed. The hybrids are uniformly self-fertile.

The methods of hybridization used need not be considered here, because they have been described in detail by Goodspeed (1912) elsewhere in this series. The particular refinements of technique which must be employed in sowing the seed, on account of its very small size, have also been there described. It might be well to state, however, that the most refined methods doubtless will not prevent the occasional appearance of a stray plant in the cultures. The danger of contamination arises not only during the sowing of the seed, but also when the bags are placed over the unopened buds. It is very easy to include a few stray seeds under the bag, for their small size makes it almost impossible to detect them in the coarse, sticky indumentum of the plant. In spite of these obvious difficulties, however, the number of plants that have certainly been strays has been very small. Their rare occurrence indicates clearly that the technique employed has been very successful.

III. ANGUSTIFOLIA–MACROPHYLLA SERIES

This series has received the most attention since the parents are so distinctly different, and the results have consequently been more complex than those which have followed the crossing of any other pair of *Tabacum* varieties. As will be demonstrated below, F_2 seemed at first hopeless in its variety of segregation. Later generations, however, exhibited so much less, or so little variety in their segregation products that it was easy to obtain new permanent combinations of characters or "fixations." Certain of its segregants have been followed out to F_7, and have also been crossed back on the parents which they most closely resembled.

Six successful crosses were made. Of these H_2 and H_3 had *macrophylla* for the male and *angustifolia* for the female parent, while H_4, H_5, H_{15}, and H_{16} were reciprocal crosses. As a matter of convenience

the generations later than F_1 were grown only from H_2 and H_4, the larger number from H_4. The predominance of H_4 in the later families selected for the continuation of the work was not, however, due to any especially different behavior evidenced in that particular series.

1. PARENTS OF THE ANGUSTIFOLIA–MACROPHYLLA SERIES

By selecting *angustifolia* and *macrophylla* for crossing, two varieties were obtained which resemble each other in height and general habit, but which differ strikingly in leaf and flower characters. The differences are sufficiently great to lead one to regard them as belonging to different species; in fact, all five *Tabacum* varieties selected by us as possibly fundamental differ sufficiently among themselves to be regarded as species in the *Tabacum* section rather than as varieties. It is not our intention, however, to emphasize this point, since any discussion would of necessity lead to a general survey of all the known varieties and races at present included under *Tabacum*. If, however, these five, viz., *angustifolia, macrophylla, "Cavala," Maryland,* and *"Brazilian"* (cf. Setchell, *loc. cit.*) could be considered by themselves as wild plants, it seems to us that any taxonomist of the present day would certainly award to each of them the rank of a separate species. These considerations should be borne in mind in estimating the significance of the results obtained through crossing.

Angustifolia, U. C. B. G. 68/07, is a variety which has long been known and which is represented in our breeding experiments by a pure line very closely approximating the type. It has been figured and discussed by one of us (Setchell, *loc. cit.,* p. 9, pl. 7). The photograph given there is of a young plant just coming into flower and consequently does not represent the habit of the plant in full blossom or in fruit, after the full number of laterals is developed. A plant in the height of its vigor is represented in plate 55, figure 1. In stature *angustifolia* belongs to the low corymbose group of *Tabacum* varieties, which also includes the forms bred in the University of California Botanical Garden under the names *calycina* and *macrophylla,* and which is in decided contrast to the tall, more "racemose" (although these may be "corymbose" at the top) forms such as *alba* and *virginica.*

In height *angustifolia* varies from 75 to 120 cm. The central axis develops its corymbose panicle of short racemes first, but it is usually soon overtopped by the successive laterals developed basipetally, each

lateral, in turn, developing a corymbose cluster of racemes, rising more or less above its predecessors. The result is that the whole plant has the short corymbose habit mentioned above. The stems and branches of *angustifolia* are comparatively slender, being much more slender than those of *macrophylla*, or those of any other of the *Tabacum* varieties except those of *calycina*, which are very similar.

The leaves of *angustifolia* are alternate and distinctly and moderately long petiolate. The blade of the lower leaves is ovate-lanceolate, tapering above to a long, curved point, more or less conduplicate below and with the rounded bases unequal. Above, the leaves are less conduplicate, more so even at the base, with the petiole shorter, while the uppermost (bracts) become almost sessile and narrowly lanceolate even to almost linear in outline. The normal petiole is naked at the base and in the middle portion, but the base of the blade is slightly and narrowly decurrent along the upper portion. Occasionally a petiole shows a narrow wing throughout its length and at times the petioles of all the leaves on certain plants are more or less winged, but the majority of the plants have naked petioles (cf. also Goodspeed and Clausen, 1917, p. 306, pl. 46, right-hand figure). The leaves of *angustifolia* have also a very characteristic drooping habit, much more pronounced than in any other *Tabacum* variety except *calycina*. In older plants, after capsule formation has well advanced, all the leaves are hanging obliquely downwards.

The flower of *angustifolia* is distinctive and differs in details of shape and color from that of any other *Tabacum* variety, and especially from that of *macrophylla*. The general shape of the flower is that of all the *Tabacum* section, but the corolla is much more slender and more gradually infundibuliform than that of any of the other varieties reported here. The calyx is broadly campanulate, prolonged above into 5 long, but unequal, linear-lanceolate, pointed lobes, of which one is longer than the remaining four and gives the calyx a zygomorphic appearance. The corolla is narrow and tubular below the middle, expanding rather gradually and evenly above into a conical infundibulum which bears the spreading, deeply 5-lobed limb at its summit. The length of the tube of the corolla is about 6 cm. and its greatest diameter about 7 mm. The limb of the corolla, at first erect (opening bud), then horizontal, finally becomes somewhat deflexed and measures about 3 or 3.5 cm. across. It is divided almost to the tube into 5 lobes which are ovate-lanceolate with long, narrow, tapering tips. The lobes of *angustifolia* are much longer and have narrower tapering tips than

those of any other *Tabacum* variety, and in this respect are in direct contrast to those of *macrophylla*. The lobes are unequal and give a slight suggestion of zygomorphism to the corolla. The stamens are inserted on the lower portion of the tube and are usually slightly exserted in anthesis. The pistil possesses the usual 2-celled ovary, long, slender style, and thick, slightly bilobed stigma, more or less exserted in late anthesis, characteristic of the genus *Nicotiana*. The color of the corolla is a light, though lively, pink, much lighter than the red of *macrophylla*. The capsule at maturity is slightly flattened longitudinally, is broadly lanceolate in profile, tapers above into an acuminate apex, and is about 25 mm. high and 8 to 9 mm. thick. It is the most slender of all the capsules borne by the various *Tabacum* varieties and in decided contrast to the stout capsule of *macrophylla*.

In plate 55, figure 1, is illustrated a plant of *angustifolia* at the height of its blooming period. Typical features of the plant are shown in the line drawings of plate 56. Photographs of typical leaves are shown in plate 58, where they may be compared with photographs of the leaves of *macrophylla*. Photographs of the flowers are reproduced in plate 60, where they may be compared with those of *macrophylla* and of the hybrids between these two varieties.

Macrophylla, U. C. B. G. 22/07, has already been discussed and figured by one of us (cf. Setchell, *loc. cit.*). The original seed was obtained from Comes, but the plants do not correspond to his figures (cf. Comes, 1899, pl. VIII) either as to habit or shape of leaf. They differ also from his description in these same respects. The flower, however, agrees, and it seems best to retain for it the name under which we have cultivated it.

The habit and height of *macrophylla* are both very similar to those of *angustifolia*. The habit is low corymbose, the central axis bearing a panicle of corymbose racemes and the laterals arising one after the other bearing similar inflorescences and equaling or overtopping the central axis. The stems and branches are stouter than those of *angustifolia*, however, and this, together with the broader, more solid looking leaves which do not droop so much as those of *angustifolia*, give a mature plant of *macrophylla* a much more robust appearance than is the case with a mature plant of *angustifolia*. The plant figured in the first number of this volume (pl. 6) was young. An older plant shown herewith on plate 55, figure 2, is in full blossom and beginning to ripen its capsules, and gives a better idea of the habit of a well grown plant.

The leaves of *macrophylla* are sessile by a partially clasping base and possess two basal lobes partially clasping the stem. The general shape is obovate, the widest portion being above the middle. The leaves taper gradually to the broad clasping base below and abruptly to a narrow more or less acuminate tip above. The surfaces show the secondary veins branching at a more obtuse angle than do those of the leaves of *angustifolia*. The color of the leaves is a dark green in *macrophylla* and more of a yellowish green in *angustifolia*. In every way, then, the leaves of the two parents differ from each other as much, in fact, as do the leaves of many species.

The flowers of *macrophylla*, while of the same general type as those of *angustifolia*, differ in details of shape and color. The flowers of *macrophylla* are about 4 cm. long. The calyx is broadly ovate in profile, deeply cut into 5 broad and somewhat unequal lobes. The corolla tube is stout cylindrical (about 5 mm. in diameter) below, broadening suddenly into a stout infundibulum above (about 10 mm. in diameter). The limb is at right angles to the tube, is about 23 mm. across, and is more or less pentagonal with 5 shallow sinuses. The color of the corolla is deep red fading to an almost lilac tint after anthesis. On the limb are 5 triangular lighter areas, one having the narrow apex at each sinus and the broad base at the top of the tube. In the much darker color, in the broader tube and stouter infundibulum, and in the barely appreciable lobing of the limb, the corolla of *macrophylla* is the antithesis of that of *angustifolia*. In stamens and pistil, the flower of *macrophylla* shows little variation from that of *angustifolia*.

The capsule of *macrophylla* is broadly ovate, tapering abruptly to a mucronate tip. It is about 2 cm. high and about 1.5 cm. in diameter, contrasting very decidedly with the comparatively slender capsule of *angustifolia*.

A typical plant of *macrophylla* is shown in plate 55, figure 2. Typical features of the plant are shown in line drawings in plate 57. Photographs of leaves are reproduced in plate 58, where they may be compared directly with those of *angustifolia*. In plate 60 its flowers may be compared directly with those of *angustifolia* and with those of the hybrids.

It has seemed best to call attention to the characters of and differences between these two varieties, parents in the first set of crosses to be discussed, in order that the behavior of their hybrid progeny may be clear. In height and habit there is a close agreement, but in leaf, flower, and fruit there are sufficient differences to mark them as separate species.

2. F_1 OF THE ANGUSTIFOLIA–MACROPHYLLA SERIES

In late July of 1909, some 7 crosses were made between *angustifolia* and *macrophylla,* 6 of which, as stated above, were successful. H_1, H_2, and H_3, as they were designated, involved *angustifolia* as the female plant, while H_4, H_5, H_{15}, and H_{16} were reciprocals. No seed was obtained from H_1, but all the other 6 crosses gave a fair yield. The usual care (cf. Goodspeed, *loc. cit.,* pp. 129–131) was taken in cleaning and sowing the seed. This was done in the spring of 1910, germination was good in all cases, and 337 plants, distributed as follows, came to maturity and seed bearing. The family of H_2 had 56, H_3 had 60, H_4 had 47, H_5 had 58, H_{15} had 55, and H_{16} had 61 plants.

A survey of all these plants showed in general a remarkable uniformity in habit. A certain amount of difference was to be detected on careful scrutiny, but little if any greater than that which is exhibited among a large number of individuals of one or the other parent. In height, F_1 showed exactly the same variation as the parent, the central axes varying from 65 to 145 cm., but largely varying from 90 to 120 cm., while the laterals rose to 150 cm. Some rows showed uniformly higher, others uniformly lower plants, the differences probably being due to different soil and water conditions. The habit (see pl. 61) was low corymbose and the general appearance as to stoutness seemed more or less intermediate between the two parents. The leaves in shape, size, etc., very closely resembled those of *angustifolia.* There was some appreciable variation in the leaves, however, and often considerable variation on the same plant, a characteristic of *angustifolia* which has already been mentioned. Plate 59 reproduces photographs of different types of leaves obtained from F_1 plants. The blade is broadly elliptical ovate with the lateral veins at an obtuse angle, much as in *macrophylla.* The base is rounded, or even slightly cordate in some leaves, while the tip is more blunt. These characters seem, at least, to indicate an influence of *macrophylla.* The leaf, however, is distinctly petiolate, but the petiole is not so long as in *angustifolia.*

The petiole is definitely winged and the wings are expanded at the base into auricles, which are often triangularly decurrent along the internode of the stem. This wing is usually present in all the plants of F_1, but some leaf or leaves on a plant may lack it, and in some plants it is only slightly developed, or at least, is without auricles. The wing was from 5 to 7 mm. wide on some leaves.

The leaf of $10F_1H_{15}P_7$ represented in plate 62 even more closely resembles the typical leaf of *angustifolia.* The wing along the mar-

gins (or edges) of the petiole is narrow and is prolonged as a slight ridge to the internode and is decurrent (?) or can be traced as it bends sharply downwards. Such wings are, at times, found in pure bred *angustifolia* plants. This leaf, then, resembles the *angustifolia* leaf fairly closely, but differs from its ordinary expression in the more tapering base, in being less distinctly conduplicate, in tapering more abruptly toward the tip, in having shorter petioles and in having more of a wing on the margins of the petiole.

The flower of F_1 (see pl. 60) resembles that of *angustifolia* more than that of *macrophylla*. The color is deep pink, decidedly of a deeper shade than is the flower of *angustifolia*, yet far from the red of *macrophylla* and in a way intermediate between the two. There is no trace, on the limb of the corolla of F_1, of the 5 white triangle-shaped areas so characteristic of the limb of the corolla of *macrophylla*. The infundibulum, while possibly slightly stouter than that of the flower of *angustifolia*, is not so stout as that of the flower of *macrophylla*. In length the flower of F_1 averages about 4 to 4.5 cm. as against an average of 6 cm. in *angustifolia*, and of 4 cm. in *macrophylla*. The tube averages about 3.5 to 5 mm. in diameter below, as contrasted with 2.5 to 3 mm. as an average in *angustifolia* and 5 mm. in *macrophylla*. The infundibulum in the corolla of F_1, while neither abrupt nor so stout as that of the flower of *macrophylla*, is noticeably more abruptly enlarged and stouter than that of *angustifolia*. The limb of the corolla in F_1 averages 2.5 to 3.25 cm. in greatest diameter, while that of *angustifolia* averages 3 to 3.5 cm. and that of *macrophylla* averages about 2.3 cm. in greatest diameter. The lobes of the corolla in F_1 are about half the width of the limb from tube margin to extreme tip of lobe, while in *angustifolia* they are about two-thirds of this and in *macrophylla* they are only one-third or even less. The lobes, also, are decidedly broad at the base, particularly so as compared with their length. In general, then, the corolla of F_1, while closer to that of *angustifolia*, shows by its stouter tube, more abrupt and more swollen infundibulum, intermediate spread of limb, less deep lobing, shorter and broader lobes, and deeper shade of pink, definite influences of *macrophylla* also.

The capsule of F_1 is broader than that of *angustifolia*, but narrower than that of *macrophylla*. There is greater variability in horizontal diameter in the capsule of F_1. The flower and fruit of F_1, then, although no careful biometric study has been made, are intermediate between those of the two parents, yet incline more toward *angustifolia* than toward *macrophylla*.

In general, then, a survey of F_1 shows throughout a series of ten different families a uniformity of individuals as great as that exhibited in either of the parents. Some few slight differences exist among individuals both of F_1 and of the parents which may possibly be referred to lack of a completely homozygous conditon in the parents. In characters in which the two parents differ, whether in color of flower, quantitative corolla character complexes, capsule character complexes, or leaf character complexes, the F_1 hybrid exhibited throughout a character expression intermediate between that of the two parents.

3. F_2 OF THE ANGUSTIFOLIA-MACROPHYLLA SERIES

In 1911, there were selected as parents for the F_2 13 plants from H_3 and 12 from H_4. Twenty-one families of approximately 50 plants each were set out in the field. On account of the great diversity shown in these populations, it was found impossible to study individually each of the thousand plants grown; consequently particular attention was paid to only 5 families from each hybrid. The other families were gone over carefully, but nothing notably different was found in their behavior. All fifty plants survived in each family except in the last, viz., $11F_2H_4P_{43}$, where only 48 came to maturity. There were then 498 plants of F_2 under more careful observation, representing both the cross and its reciprocal, with about 550 remaining for only casual examination.

As might have been expected, there was a great variety of plants resulting and segregation as to differences in combination of characters of flower, fruit, and leaf was little short of bewildering. An attempt was made to study and arrange these combinations, but it was found to be impossible. A careful survey, however, was made of the populations and a tabulation of characters was attempted. Some 16 fairly readily separable types, based on leaf characters, were distinguished, but between these closely approaching types others were to be found of intermediate and overlapping character. One each of the types selected was drawn, and these drawings are reproduced in plates 63 to 78.

A glance at these plates, which were carefully drawn to scale, will show something of the nature of the combinations of characters of the two original parents. Type 1 (pl. 63) shows a close approximation, yet not an absolute reproduction, of *angustifolia*, while type 16 (pl. 78) in a similar way is a close approximation to *macrophylla*. The other

14 types (pls. 64 to 77) are clearly intermediates approaching one parent more than the other, but types 12, 13, and 14 (pls. 74 to 77, inclusive) are decidedly different from either as to leaf, at least, and type 10 (pl. 72) is of another altogether different form, although all of these leaf shapes are connected to a greater or less extent into one series of more or less gently intergrading forms.

As to the shape and dimensions of the corolla there is to be found a similar series of intergrading forms from the slender corolla tube with gradually expanding and slightly swollen infundibulum and deeply lobed limb of type 1 (pl. 63) to the corolla with stout tube, abruptly and considerably swollen infundibulum with slightly lobed limb of type 16 (pl. 78). In color the corollas vary from the light pink of *angustifolia* to the red of *macrophylla* and three shades are at times fairly readily distinguishable, the light pink of *angustifolia*, the deep pink of F_1, and the several nuances of the red of *macrophylla*.

The capsules also show various combinations from the slender gradually attenuated capsules of *angustifolia* to the stout, swollen, abruptly upwardly attenuated capsules of *macrophylla*. Both capsules and corollas approaching one parent may be found with leaves more closely approaching the other parent. In stature and habit the plants of all the 21 families were reasonably uniform and agreed in general in these respects with the parents and F_1, there certainly being no greater amplitude of variation in these respects than was to be found in the parental types.

Among the great variations, two characters seemed to stand out fairly clearly for rough statistical enumeration, viz., color of the corolla and the possession, or lack, of a petiole. Numerical data for these characters are given in table 1. Some care was taken to obtain a careful census of the families as regards each of the characters. As regards colors, it was, as noted before, possible to distinguish three shades, or sets of shades, which were designated as light pink, pink, and red. In practice, however, it was usually difficult to distinguish the two shades of pink from each other. The red gave very little trouble.

In attempting to classify the plants of F_2 with respect to type of leaf base, more difficulty was experienced because of the variety of forms which were produced and the degree of intergradation which existed between forms. In judging the presence or absence of petiole, therefore, in these populations, the classification is faulty because of lack of knowledge of the genetic constitution of the various distinct forms and those which grade into them. In table 1 the plants are

thrown into the petiolate class if they were distinctly narrowed at the base, and whether naked or winged.

In F_2, then, there appears to be simple Mendelian inheritance in only one pair of the original character contrasts of the parents, namely, red versus pink corolla color. Here the hybrid is intermediate and F_2 segregates sharply into pink and red in the ratio 3 pink : 1 red. Within the pink class there is a more or less evident segregation into 2 pink : 1 light pink, but the shades intergrade so that no distinct line of demarcation exists between the classes. As respects leaf base characters, the segregation is so complex that no reasonable genetic analysis is possible. The numerical data for this latter character presented in table 1 are of value only in that they indicate a close agreement in segregation among F_2 families, thereby furnishing a rough statistical demonstration of the equivalence of the several families. The more definite data on leaf base characters are derived from generations subsequent to F_2.

TABLE 1

CLASSIFICATION OF F_2 PLANTS OF THE *ANGUSTIFOLIA-MACROPHYLLA* SERIES ACCORDING TO COROLLA COLOR AND LEAF BASE CHARACTERS.

Family Designations	Garden Numbers	Corolla Color			Leaf Base	
		red	pink	light pink	petiolate	non-petiolate
A	$11F_2H_2P_2$	12	23	14	42	7
B	$11F_2H_2P_3$	14	25	10	30	20
C	$11F_2H_2P_6$	13	21	15	34	14
D	$11F_2H_2P_7$	7	21	22	35	15
E	$11F_2H_2P_{13}$	15	20	14	30	20
F	$11F_2H_4P_2$	13	28	7	32	17
G	$11F_2H_4P_{35}$	12	29	8	35	14
H	$11F_2H_4P_{40}$	13	15	22	32	18
J	$11F_2H_4P_{41}$	8	31	10	31	17
K	$11F_2H_4P_{43}$	6	27	15	38	10
Totals		113	240	137	339	152

4. F_3 AND SUBSEQUENT GENERATIONS OF THE ANGUSTIFOLIA–MACROPHYLLA SERIES

From F_2 of H_2 and H_4, 20 plants were selected for further experimentation and families of 25 were determined upon as the unit. In all except four, the families of 25 each were successfully raised. Of one of the four only 14 plants were obtained, which were all that germinated, while in each of the other three families 24 plants were reared to maturity. Altogether, then, 486 plants were raised of the F_3 during the season of 1912. It was the intention to grow from each of the selected types, as drawn for illustration. Fifteen of the families from the type parents were successfully reared, but, in some way or other, the seed of type 4 ($10F_2H_2P_7P_{18}$) was not to be found, and, unfortunately, no note had been made as to whether or not any seed was produced. No complete sterility, however, was noticed in any members of F_2 of either H_2 or H_4, and the presumption is that F_2 seed of type 4 must have been lost in harvesting.

The variation within each family was decidedly less than that of the families of F_2. Of the 20 families reared wholly or in part, 4 families were very nearly uniform, varying in minor details only. Five families segregated only in corolla color, 4 segregated only in leaf base characters, and the remaining 7 segregated both in corolla color and leaf base characters. In table 2 are summarized the data as to gross behavior of these families of F_3. In those families grown in F_4 and subsequent generations, a definite attempt was made to fix the original characters of the F_2 type selection in a pure line. The genealogical relation of these selected lines to each other is shown in the chart reproduced herewith. The letters, A, B, C, etc., correspond to the F_2 family designations noted in table 1, and the numbers refer to type selection numbers corresponding to the type illustrations in plate 63 to 78, or, in the case of types 17 to 21, corresponding to those types as described in the succeeding accounts of the later generations.

TABLE 2

F_3 FAMILIES OF THE *ANGUSTIFOLIA-MACROPHYLLA* SERIES.

Type Nos.	Garden Numbers	No. of plants in families	Results in F_3
1	$11F_2H_2P_7P_{49}$	25	Segregated both as to leaf and flower color
2	$11F_2H_2P_3P_{30}$	25	Segregated both as to leaf and flower color
3	$11F_2H_2P_3P_{14}$	14	Segregated only as to leaf
5	$11F_2H_4P_{41}P_{14}$	24	Segregated both as to leaf and flower color
6	$11F_2H_4P_2P_{18}$	25	Uniform except as to length and development of wing of petiole
7	$11F_2H_2P_{13}P_{48}$	25	Uniform both as to leaf and flower color
8	$11F_2H_2P_3P_{41}$	25	Segregated as to leaf only
9	$11F_2H_4P_{41}P_8$	25	Segregated both as to leaf and flower color
10	$11F_2H_4P_{41}P_{17}$	24	Segregated only as to flower color
11	$11F_2H_4P_{41}P_9$	25	Segregated only as to flower color
12	$11F_2H_4P_{41}P_{12}$	25	Segregated only as to flower color
13	$11F_2H_2P_3P_{44}$	25	Segregated only as to flower color
14	$11F_2H_2P_3P_{38}$	25	Segregated both as to leaf and flower color
15	$11F_2H_2P_3P_{10}$	25	Uniform, slight variation in tint and lobing of corolla
16	$11F_2H_2P_3P_8$	24	Segregated only as to leaf
17	$11F_2H_4P_{35}P_{27}$	25	Segregated only as to flower color
18	$11F_2H_4P_{35}P_{38}$	25	Segregated both as to leaf and flower color
19	$11F_2H_4P_{35}P_{43}$	25	Segregated both as to leaf and flower color
20	$11F_2H_4P_{40}P_{44}$	25	Uniform, close to *macrophylla*
21	$11F_2H_4P_{41}P_{29}$	25	Uniform, close to *angustifolia*

In F_2 primary selection for parents of subsequent generations was based upon the type of leaf borne by the plant, flower color being followed as a secondary matter. In order to systematize the discussion concerning F_3 and subsequent generations, six general types have been selected and named and the discussion of the families has been grouped

Fig. 1.—Chart showing the relationships of the various families of the *angustifolia-macrophylla* series. The different F_1 hybrids are connected with their female parents; no seed was secured from H_1. The F_2 family designations correspond to those given in table 1, and the numbers in later generations are the type numbers under which the populations are described in the text. F_4 and F_5 of type 12a were grown in 1914 and 1915 respectively.

under these headings. The six general types selected and the names given them are as follows:

a. Stenophylla derivatives. As a series these approximate very closely in leaf shape to *angustifolia.* The distinguishing feature of this series is the possession of a distinct, long petiole.

b. Latifolia derivatives, which are characterized by the possession of a broad leaf with a petiole shorter than that of *angustifolia.* The petiole in these types is more or less winged.

c. Lanceolata derivatives, which are characterized by the possession of a lanceolate leaf like that of type 13, illustrated in plate 75. This is a non-petiolate form.

d. Loriifolia derivatives, characterized by possession of long leaves with very narrow blades. The type specimen, type 12, is illustrated in plate 74. This also is a non-petiolate form resembling the lanceolata derivatives, from which it differs in the extreme narrowing of the blade.

e. AURICULATA derivatives. The typical form of leaf is that of type 10, illustrated in plate 72. The leaf blade of this form is characterized by an abrupt contraction of the blade at the base, nearly, if not quite, to the midrib. Clasping auricles, from which the name is derived, are usually present in this form.

f. SESSILIFOLIA derivatives, of which the leaf of *macrophylla* may be taken as the type. These derivatives are non-petiolate, as the name indicates.

a. STENOPHYLLA derivatives

Type 1, as may be seen from the drawing of F_2 (cf. pl. 63), seems very close to *angustifolia,* and had light pink flowers. There were 25 plants of $12F_3H_2P_7P_{49}$, the leaves of which were carefully noted; but one passed its flowering stage too early to be judged, so that the colors of the flowers of 24 only are known. Sixteen plants had STENOPHYLLA leaves very nearly of type 1, but the extent of the wing structure varied somewhat. The remaining 9 had LANCEOLATA leaves of type 14. In flower color the segregation ratio noted was 5 red : 19 pink. No further generations of this line were grown.

Type 17 was not selected for illustration in F_2, but was a plant very close to *angustifolia.* It had, however, somewhat narrower leaves and deep pink flowers. The 25 plants of $12F_3H_4P_{35}P_{27}$ were uniform and like the F_2 parent except as to flower color; 3 were red and 22 were deeper or lighter pink. This line was not grown through further generations.

Type 21, also, was not selected for illustration of the F_2 plant, but was chosen later for perpetuation because of its extremely close agreement with *angustifolia.* The F_3, $12F_3H_4P_4P_{29}$, consisted of 25 vigorous plants which seemed to be uniform to the finest detail and agreed in every respect with plants of *angustifolia.* There were noticed in the plants of this family peculiar fimbriae attached to the corolla, or split corollas, or, in one instance, a split hose-in-hose flower such as occurs at times also in pure *angustifolia.* This type seemed to be a pure recombination of the characters of *angustifolia.*

Type 21 was continued through to the seventh generation and found to be constant in the uniformity of the individuals in the several families. In 1913, 94 plants of F_4 were grown; in 1914, 85 plants of F_5; in 1915, 10 plants of F_6; and in 1916, 8 plants of F_7. All these populations closely resembled one another as to individuals as well as those of the F_3 and the F_2 parent. They are all so close to *angus-*

tifolia as to be practically indistinguishable from it. This line may be regarded, therefore, as a stable derivative very closely approximating *angustifolia* in all its characters.

b. Latifolia derivatives

Type 2 (cf. pl. 64) approached in F_2 fairly near to some of the variations of F_1 (cf. pl. 62). It might be regarded as a stenophylla derivative possessing an unusually luxuriant development of the wing of the petiole, but it seems more reasonable to classify it as a latifolia derivative exhibiting marked narrowing at the base of the lamina, such as is shown in lanceolata derivatives. The flower was designated as light pink in the notes taken at the time of flowering. The leaves of F_3 showed segregation through a considerable range, 6 being close to the latifolia type of F_1, 6 to the latifolia type of its F_2 parent (type 2), 8 were sessilifolia leaves approaching in type those of *macrophylla*, and 4 were auriculata leaves of type 10. In 13 the flowers were noted as light pink, in 11 as pink, and in 1 as red. No subsequent generations of this population were grown.

Type 3, in F_2, had (cf. pl. 65) a distinctly ovate lanceolate leaf with a short fairly broadly margined petiole. The flowers were light pink. F_3, $12F_3H_2P_3P_{14}$, amounted to 14 germinations, all of which developed, although slowly, into healthy, normal appearing plants. The flowers were all the light pink color of the F_2 parent, agreeing with those of *angustifolia*, but varying somewhat in shape and size. The leaves were of various shapes, 4 were distinctly petiolate, while 10 were sessile. Of the 4 petiolate plants the petiole of 1 was naked and of 3 more or less winged. Of the 10 sessilifolia plants, 9 were very similar to type 11, but 1 was rather longer and narrower, although otherwise approaching the same general shape. No further generations from this line were grown.

Type 5 in F_2 (cf. pl. 67) resembled F_1 most nearly, but the winged petiole was short and the corolla tube slightly stouter. The flower color was pink. Of $12F_3H_4P_{41}P_{14}$, 24 plants were grown. Of these 23 had leaves almost exactly like those of the F_2 parent, but 1 had leaves more nearly like auriculata of type 8 (cf. pl. 70). Of the 24 plants, 16 had flowers of various shades of pink while 8 had red flowers. This line was not grown in subsequent generations.

Type 6 was represented in F_2 by a plant which resembled F_1 in having a winged petiole to the leaf and a pink flower. It is well repre-

sented on plate 68. In the 25 plants of $12F_3H_4P_2P_{18}$, the height, habit, flower shape, and flower color were close to, if not identical with, those of the F_2 parent. As respects leaf base characters, 14 were LATIFOLIA of type 6, 4 had long, naked petioles, 3 had short, naked petioles, 1 had a long, winged petiole, and 4 were AURICULATA plants nearly of type 10. This seems like a considerable segregation, but the leaves are of only two generic types, viz., petiolate and non-petiolate. In subsequent generations selection was made in one line for LATIFOLIA leaves of type 6, and in the other for AURICULATA leaves of type 10, called type 6a to indicate its derivation.

Of type 6 as thus established 100 plants of F_4 were grown in 1914; 100 plants of F_5 (50 each from 2 different parents) in 1915; 20 plants of F_6 in 1915; and 20 of F_7 in 1916. All the individuals thus grown were constant to LATIFOLIA of type 6 as originally selected.

Of AURICULATA of type 6a, similarly, 100 plants of F_4 were grown in 1913; 94 of F_5 in 1914; 20 of F_6 in 1915; and 20 of F_7 in 1916. All these plants were uniform and true to AURICULATA of type 6a, very close to AURICULATA of type 10. In both these types we have definitely obtained stable recombinations of germinal elements exhibiting characters different from those of the parents.

F_3 of type 7, $12F_3H_2P_{13}P_{48}$ (cf. pl. 69), consisted of a family of 25 plants, all vigorous except one (P_{17}), which was set out in the field later and developed into a "runt," as often happens with such later plantings. All the plants agreed well with one another in height and habit except the "filler," and all agreed in inflorescence, flowers, and leaves. There were some variations in size and lobing of the limb of the corolla, indicating possibly minor segregation, but in all general characters there was uniformity to a large degree. The plants agreed well in all characters with the F_2 parent, and also with the F_1 parent. The color of the flower was light pink, the petioles of the leaves varied somewhat in length, were distinctly and more or less broadly winged, and the blade was heart-shaped, at least at the base in the lower leaves. In all respects these characters were no more variable than they were found to be in F_1.

Type 7 continued to breed true in subsequent generations. It was grown in 1913 (100 plants, F_4), 1914 (2 families of 50 plants each, F_5), 1915 (10 plants, F_6), and 1916 (10 plants, F_7). All were uniform as to leaf and flower color. Type 7 is very close to the type of F_1 and to type 6 described above. It, too, evidently represents a stable recombination of germinal elements derived from both parents.

Type 9 resembles type 5, but had in F_2 a very short winged petiole and elliptical lanceolate blade. It also had pink flowers. F_3, $12F_3H_4P_{41}P_8$, consisted of 25 plants, 18 of which showed LATIFOLIA leaves of type 9, but 7 had SESSILIFOLIA leaves of type 14 (cf. pl. 76). Twenty-one had pink (or light pink) flowers and 4 had red. No further generations of this line were grown.

Type 19 was an F_2 plant of which no drawing was made, but it resembled F_1 (cf. pl. 62), having broadly ovate leaves with a long and broadly winged petiole and pink flowers. F_3, $12F_3H_4P_{35}P_{43}$, consisted of 25 plants, 6 of which had SESSILIFOLIA leaves of type 16 (cf. pl. 78) or nearer, perhaps, to those of *macrophylla*, while 19 had LATIFOLIA leaves of type 19. In 5 plants the flowers were a somewhat darker red than they were in the other 20. This line was not followed further.

c. LANCEOLATA derivatives

Type 13 is similar to type 12 described below, but the leaves of the F_2 plant were more lanceolate and broader and the flowers were lighter pink. The 25 plants of $12F_3H_2P_3P_{44}$ were uniform and like F_2 except in flower color. Four were red, 19 decidedly pink, and 2 inclined to light pink. The line was not grown in subsequent generations.

d. LARIIFOLIA derivatives

Type 12, as shown in plate 74, differed very decidedly in leaf shape from either parent. The long linear-lanceolate leaf had the long tapering curved tip of *angustifolia*, but the blade tapered below, making practically a new type. The flowers were like those of *angustifolia* in shape but were pink. The 25 plants of F_3, $12F_3H_4P_{41}P_{12}$, were exact duplicates of F_2 as to habit, leaf, and flower shape, but 10 had red and 15 had pink flowers of various shades, mostly dark. None seemed as light pink as *angustifolia*.

This is the most interesting of the types carried through subsequent generations, representing, apparently, a new combination of leaf characters. One of the pink flowering F_3 plants was chosen for seed and the designation, type 12, retained for this and its progeny, while the designation, 12a, was given to one of the red flowering F_3 plants also chosen for seed.

Type 12, as thus limited to the pink flowered plant, gave scanty germination and few plants for F_4 in 1913. Apparently it was still varying slightly in color within the pink shades, although fairly uni-

form except for one aberrant (?) plant of a decidedly lighter shade. Two "normal" parents of F_4 gave 88 and 100 plants of F_5 in 1914, which were uniform and of a bright pink color. In 1915 F_6 showed 10 plants, and in 1916 F_7 also showed 10 plants, still uniform and pink.

Type 12a on being segregated in the second growing of F_3 in the season of 1913 yielded 100 plants of F_4 in 1914, uniform and of deep red flower color. F_5, of 10 plants in 1915, and also F_6, of 10 plants in 1916, produced uniform individuals of deep red flower color.

We find, then, in types 12 and 12a definite fixations of the LORIFOLIA type, one with uniformly pink flowers and one with uniformly deep red flowers.

e. AURICULATA derivatives

Type 8 is represented in F_2 by a plant which had a leaf with an extremely constricted base (cf. pl. 70) and deep red flowers. It is not a typical AURICULATA derivative, but is included under this heading because it resembles the members of this class more closely than those of any other. F_3, $12F_3H_2P_3P_{41}$, consisted of 25 plants which were uniform in height, habit, and flower color, and in agreement with F_2 in these respects. The leaves, however, were of two distinct types, 16 AURICULATA of type 8 and 7 SESSILIFOLIA of type 16 (cf. pl. 78), the latter being near to the type of *macrophylla*. No further generations of this line were grown.

Type 10, as shown by the drawing (pl. 72), had a peculiar leaf, near to the *macrophylla* type, yet deeply constricted at the base into a narrow and extremely abbreviated structure which may resemble a petiole or only a deeply constricted blade. There were, however, auricles partially clasping the stem and slightly decurrent. The leaf form was that characteristic of *N. Tabacum* var. *macrophylla purpurea* (cf. Setchell, *loc. cit.*). All 24 plants of F_3 had the same type of leaf as F_2, but the flowers were of three fairly readily distinguishable shades; 3 were red, 16 pink, and 5 light pink. F_2 had very dark pink flowers. The line was not grown in further generations.

Type 6a is a true AURICULATA derivative which segregated in F_3 from an F_2 LATIFOLIA selection. Its occurrence and behavior are described in connection with the account of type 6, the LATIFOLIA type from which it segregated. Grown in the pure line for five generations it has remained constant for the AURICULATA type of leaf.

f. Sessilifolia derivatives

Type 11 (cf. pl. 73) in F_2 gave 25 plants in $12F_3H_4P_{41}P_9$, all vigorous except one, but that one showed the same characters of leaf and flower as the others. All 25 plants possessed a sessilifolia type of leaf very close to the F_2 parent and uniform among themselves. There were two distinct shades of color of the flowers, 9 red and 16 pink. No further generations of this line were grown.

Type 14 (cf. pl. 76), so far as F_2 is concerned, was one of those having sessile leaves of a broadly lanceolate type and pink flowers. There were 25 plants in $12F_3H_2P_3P_{38}$, 24 had sessilifolia leaves of type 14, while one (a "filler") had auriculata leaves like type 8; 19 had pink (or light pink) flowers, while 6 had red flowers. This line was not followed through subsequent generations.

Type 15 (cf. pl. 77) was represented in F_3, $12F_3H_2P_3P_{10}$, by 25 vigorous plants which seemed surprisingly uniform and approached *macrophylla* very closely as to leaf and color of the flower. In the flower, however, the color seemed even darker than that of *macrophylla*, there were only slight traces of the white triangular markings on the limb, the limb was much more deeply lobed, and the tube less stout and with the infundibulum much less abruptly swollen. These differences seem to indicate that type 15, which all the F_3 plants closely resemble, is not an exact recombination representing *macrophylla*.

Type 15 was represented in 1913 by two families, F_3 of 10 plants and F_4 of 100 plants. Both families were uniform as to individuals, and agreed with the F_3 population grown in 1912 as well as with the F_2 ancestor of the season of 1911. As this line seemed to be constant and very close to, although not absolutely identical with, *macrophylla*, differing in flower shape and leaf shape to some extent, type 15 was considered to be a fixation and no further cultivation of it was made.

Type 16 (cf. pl. 78), which in F_2 approached *macrophylla* very closely in leaf, flower shape, and flower color, was represented in F_3, $12F_3H_2P_3P_8$, by 25 plants. These were all alike and closely resembled the F_2 parent in all respects except in leaf shape. Fifteen had sessilifolia leaves of type 16 while 8 had auriculata leaves approaching those of type 10 (cf. pl. 72). This line was not grown in further generations.

Type 18 is the designation given to an F_2 plant, of which no drawing was made. It seemed close to *macrophylla*, but the flower color

was pink and the leaves were more slightly attenuate at the base. F_3, $12F_3H_4P_{35}P_{38}$, gave 25 plants, 13 of which had the SESSILIFOLIA leaf of type 18; 7, AURICULATA of type 10; and 5, AURICULATA of type 8. In flower color, 17 were some shade of pink and 8 red. The line was not grown in further generations.

Type 20 was not selected for illustration in F_2, but was a plant chosen because of its very close resemblance to *macrophylla*, coming even closer than type 16. The F_3, $12F_3H_4P_{40}P_{44}$, consisted of 25 vigorous plants of remarkable uniformity. In height, habit, inflorescence, flower, color, shape, fruit, etc., the details follow those of *macrophylla* so closely as to be indistinguishable unless possibly by careful and laborious biometric study. This type may represent a practically pure recombination equivalent to *macrophylla*, and is to be compared and contrasted with type 15.

In 1913 two families of F_4, one of 21 plants and the other of 100 plants, were uniform, as were 3 families of 50 plants each of F_5 in 1914. In 1914, however, a surprising thing happened. A fourth family of F_5, consisting of 50 plants, was uniform except one plant which had pink (instead of red) flowers and an AURICULATA leaf approximating type 8 or 10. It seems certain that this plant must have been an intruder, but its seed was saved under bag and grown and is noted below and on the pedigree chart as type 20a. The other 3 plants of F_5 whose seed was sown in 1915 gave type 20 in F_6 in families of 10, 9, and 8 respectively, and in turn the seed of 4 individuals of "pure" type 20 gave, in 1916, uniformity in families of 10 each.

Type 20a, which originated or intruded in 1914, in one plant of F_5 of type 20 gave in F_6, in 1915, 10 plants segregating for flower color and probably also for leaf characters, although the notes taken are inconclusive on the latter point. In 1916 F_7 of 10 good plants showed uniformly red flowers, but 7 had SESSILIFOLIA leaves, 4 of which were decidedly contracted at the base and 3 had very short winged petioles (AURICULATA of type 8 or type 10). On the whole it seems most likely that the single plant in the F_5 family was an intruder, since all other families of the line have been constant since F_2. A stray seed somewhere along the processes of culture would explain it and its appearance is all the more incomprehensible as a matter of inclusion in the pedigree of type 20, as it is so close to *macrophylla* as to seem practically identical with it.

5. SUMMARY OF FLOWER COLOR OBSERVATIONS IN F_2 AND SUBSEQUENT GENERATIONS

In tables 3 and 4 we have summarized the numerical data with respect to flower color inheritance in F_3 and in the subsequent populations. In table 3 are assembled data with respect to the behavior of red flowering selections from populations segregating for red and pink. It will be noted that all the five selections which were made bred true for red flower color in the succeeding generations. In table

TABLE 3

INHERITANCE OF RED FLOWER COLOR IN F_3, *et seq.*

Type Numbers	Garden Numbers	Flower color of population
8	$12F_3H_2P_3P_{41}$	25 red
12a	$14F_4H_4P_{41}P_{12}P_1$	100 red
15	$12F_3H_2P_3P_{10}$	25 red
16	$12F_3H_2P_3P_8$	24 red
20	$12F_3H_4P_{40}P_{44}$	25 red

TABLE 4

POPULATIONS FROM PINK FLOWERING SELECTIONS OF ALL SHADES IN F_3 *et seq.*

Type Numbers	Garden Numbers	Parent Color	Flower color classification		
			red	pink	light pink
3	$12F_3H_2P_3P_{14}$	light pink	....	....	14
6	$12F_3H_4P_2P_{18}$	pink	....	25	....
7	$12F_3H_2P_{13}P_{48}$	light pink	....	....	25
12	$13F_4H_4P_{41}P_{12}P_8$	pink	....	88	....
12	$13F_4H_4P_{41}P_{12}P_9$	pink	....	100	....
21	$12F_3H_4P_4P_{29}$	light pink	....	....	25
1	$12F_3H_2P_7P_{49}$	light pink	5	19	(or light pink)
2	$12F_3H_2P_3P_{30}$	light pink	1	24	(13 light pink)
5	$12F_3H_4P_{41}P_{14}$	pink	8	16	(or light pink)
9	$12F_2H_4P_{41}P_8$	pink	4	21	(or light pink)
10	$12F_3H_4P_{41}P_{17}$	pink	3	21	(5 light pink)
11	$12F_3H_4P_{41}P_9$	pink	9	16	(0 light pink)
12	$12F_3H_4P_{41}P_{12}$	pink	10	15	(0 light pink)
13	$12F_3H_2P_3P_{44}$	pink	4	21	(2 light pink)
14	$12F_3H_2P_3P_{38}$	pink	6	19	.....
17	$12F_3H_2P_4P_{35}P_{27}$	pink	3	22	.....
18	$12F_3H_4P_{35}P_{38}$	pink	8	17	.....
19	$12F_3H_4P_{35}P_{43}$	pink	25	....	(5 darker red)
Totals of segregating populations			60	187	(Types 2 and 19 excluded)

4 are assembled the data from pink flowering selections from populations which showed segregation into red and pink. In this table the populations which bred true for pink are assembled in the upper portion of the table, and those which showed further segregation into red and pink are assembled in the lower portion. Of the 18 selections made, 7 bred true for pink (or light pink), and 10 gave segregation in the succeeding generation in about the ratio of 3 pink : 1 red. The total figures for the 10 populations—187 pink : 60 red—are in very satisfactory agreement with the simple Mendelian ratio. The family of type 19 behaved in an anomalous fashion, which may indicate misclassification of the F_2 parent; and the family of type 2, which showed only one red plant has been included among those which bred true for pink. Strictly light pink selections should have given only light pink flowers in subsequent generations; the pink ones should all have given segregating populations. The evidence indicates that this result would be obtained if segregation occurred for *only one pair of allelomorphs.* The difficulty, in part at least, appears to be the result of segregation of modifying factors in the populations. These factors apparently have an effect on flower color sufficient to obscure segregation into pink and light pink, but not enough to obscure the segregation into red and pink. The actual results indicate an approximate agreement with expectation, but the breeding test clearly is necessary in order to determine the actual distribution of the pink individuals into their genetic classes.

6. LATER SOWINGS OF F_2 AND F_3 OF THE ANGUSTIFOLIA–MACROPHYLLA SERIES

In 1916 and 1917 certain families of F_2 and F_3 of H_2 were grown in order to reëxamine them in the light of data previously collected and to determine whether or not any more definite classifications could be made than those stated in the preceding pages. The populations grown are described briefly below.

$16F_2H_2P_6$, as the population number would indicate, was a sowing of seed of $10F_1H_2P_6$ from the original F_1 population of H_2. As in previous cases, the segregation as regards leaf shape was so complex as to preclude definite classification. The types previously noted for second generation populations were all in evidence and along with them practically every sort of intermediate. The height of plants and general habit likewise agreed with the description previously given.

It was possible as in previous instances to segregate the plants into definite flower color classes. In order to make this segregation as accurate and free from bias as possible a special method of classification was adopted. At the height of the blooming season, single typical flowers were collected from each plant of a population and placed in vials correspondingly numbered. These specimens were then taken into the laboratory, where they could be classified under optimum light conditions. The specimens so collected could then be shifted around into their phenotypic classes and properly compared with each other and with the parent colors. The color classification thus obtained was individually recorded, and later the population was checked over in the field to insure correction of any errors of classification. The surprising feature of this population was a sharp, three-class segregation into red, pink, and light pink; the reds the shade of *macrophylla*, the light pinks almost exactly that of *angustifolia*, and the pinks intermediate between the two. Within the classes there appeared to be no significant differences in depth of shade. Two plants bore no flowers. The ratio obtained was 15 red : 23 pink : 10 light pink.

$16F_2H_2P_{17}$ was likewise a sowing of the seed of one of the original F_1 plants, in this instance of $10F_1H_2P_{17}$. As respects habit, height, and leaf shape, there was a strict resemblance throughout of this population to the one described above. Flower color was studied in the same manner and with substantially the same results. However, in this population there was a shading off from pink to light pink, such that it was impossible to draw a sharp line between these two classes as was done in the previous population. The shading off was abrupt, but there were, nevertheless, a few plants on the border line. The observed ratio was 16 red : 34 pink and light pink.

In 1917 six F_3 populations, each containing approximately 100 plants, were grown in order to make further studies of the inheritance of leaf shape. It was impossible, however, to study these plants as thoroughly as might have been desired on account of conditons obtaining during 1917. However, specimens of leaves from each plant were pressed and preserved and these were studied and classified in the summer of 1919. A brief account of each population follows:

$17F_3H_2P_{17}P_6$ was a sowing from $16F_2H_2P_{17}P_6$, a STENOPHYLLA selection. With respect to leaf base characters the segregation was roughly but rather obviously into two types, a long petioled STENOPHYLLA class approximating type 1 in appearance, and an AURICULATA

class approximating type 10. Within the STENOPHYLLA class there was a variation in the amount of "wing" on the petiole and in the type of blade base, some having the abrupt base of type 1, whereas others had an attenuated type of blade which gradually drew in to the petiole. In the AURICULATA class there was also a variation from the strict form of type 10 to a type which lacked the flaring auricle typical for that form, and had a very short naked petiole. In addition to this variation in the amount of "wing" of the constricted class there was also a difference in the presence or absence of attenuation noted for the STENOPHYLLA class, some plants having leaves abruptly drawn in to the midrib, whereas others were very markedly attenuated. The difference in this respect appeared to be equivalent in the two distinct classes, i.e., it was independent of any difference in the "petioled" or "constricted" condition. With respect to STENOPHYLLA VS. AURICULATA the segregation was 66 STENOPHYLLA : 32 AURICULATA.

$17F_3H_2P_{17}P_8$ was a sowing from $16F_2H_2P_{17}P_8$, another STENOPHYLLA selection. The leaf classes obtained here were two, STENOPHYLLA (type 1) and SESSILIFOLIA (type 15). The segregation into the two classes was distinct, but, as in other populations, there was a great deal of variability in each class. There was attenuation of the kind previously noted in both classes. Some of the petioled individuals had distinct wings, but the larger number were naked. Some few individuals had very short petioles. The segregation ratio was 76 STENOPHYLLA : 24 SESSILIFOLIA.

$17F_3H_2P_{17}P_{19}$ was a sowing from $16F_2H_2P_{17}P_{19}$, a STENOPHYLLA selection. The population was remarkably uniform in leaf shape, which closely approximated *angustifolia* with minor differences. The straplike leaves which are a characteristic feature of the upper portions of plants of *angustifolia* were lacking in this population, and the leaf tip and distal portion of the leaf blade did not narrow so gradually in this population as in *angustifolia*. Otherwise, the characters of the plants throughout were closely similar to *angustifolia*.

$17F_3H_2P_{17}P_{22}$ was a sowing of seed of $16F_2H_2P_{17}P_{22}$, a STENOPHYLLA selection. Of the six F_3 populations studied, this one exhibited the greatest diversity in segregation. With respect to leaf base characters, there were two outstanding classes, STENOPHYLLA and SESSILIFOLIA (type 15), which could be separated readily. Within the STENOPHYLLA class, however, most of the individuals exhibited a more or less winged condition. Within the SESSILIFOLIA class, on the other hand, most of the individuals exhibited more or less narrowing of the leaf base, like

type 14. A few of the sessile individuals, instead of exhibiting gradual and uniform narrowing toward the base of the leaf, were constricted to a degree intermediate between AURICULATA of type 10 and SESSILIFOLIA of type 15. With respect to STENOPHYLLA versus SESSILIFOLIA the observed segregation was 67 STENOPHYLLA : 32 SESSILIFOLIA.

$17F_3H_2P_{17}P_3$ was a sowing of seed of $16F_2H_2P_{17}P_3$, an F_2 SESSILIFOLIA selection. The leaves throughout had the sessile type of leaf base characteristic of *macrophylla,* but there were many modifications of it in the population. A rough classification with respect to these modifications of the *macrophylla* type of leaf base gave the following results:

On 59 plants, the leaf bases were very nearly the form typical for *macrophylla.*

On 22 plants, the leaf bases were gradually attenuated toward the base, resembling LANCEOLATA of type 13 as a mean. This attenuated form of the sessile leaf was a very striking feature of this population.

On 10 plants, the leaf bases were intermediate in type between LANCEOLATA of type 13 and the typical *macrophylla* form.

On 2 plants, the base of the leaf immediately above the point of attachment was noticeably constricted, the leaf base thus formed being intermediate between the *macrophylla* type and AURICULATA of type 10.

On 2 plants, the leaves were intermediate in constriction of the leaf base between the strict *macrophylla* type and that of the two plants described immediately above.

The classification here given is presented only to show that the sessile type of leaf base characteristic of *macrophylla* is subject to a number of very definite modifications which probably account for some of the complex types of segregation observed in other populations.

$17F_3H_2P_{17}P_{12}$ was a sowing from $16F_2H_2P_{17}P_{12}$, a SESSILIFOLIA selection. With respect to leaf base segregation there were two distinct classes, SESSILIFOLIA (type 15) and AURICULATA (type 10). There was here also a marked degree of variation within the classes. Within the sessile class the variation was in amount and kind of narrowing of the leaf blade toward the base. A few plants showed a condition approaching the AURICULATA type in this respect, whereas others showed a gradual attenuated form of narrowing such as has been noted before in other populations. Within the AURICULATA class most of the individuals instead of possessing the slight wing and flaring

auricles of type 10 had short naked petioles. A few were strictly of type 10. The following segregation ratio was noted: 61 SESSILIFOLIA : 27 AURICULATA.

7. CROSSES OF DERIVATIVES WITH THE PARENTS

In the preceding account we have pointed out that by growing definite hybrid selections in the pure line through a number of generations it has been possible to establish a certain number of stable derivatives which represent more or less obvious recombinations of characters of the original parents. In a Mendelian sense, they represent stable reorganized germinal complexes containing hereditary elements that have been derived from both parents. Obviously such recombinations of Mendelian units must differ in fewer units from either parental type than did the parental types from each other. To test some of these derivatives we have crossed them with the original parents, usually with the one to which they bore the closest resemblance, in order to observe how complex a type of segregation the hybrids thus obtained would exhibit as compared with that of the original *angustifolia-macrophylla* hybrids. In so far as they have been studied to date, a description of these hybrids and their progenies follows:

SESSILIFOLIA×*macrophylla.* F_5 SESSILIFOLIA of type 20 was crossed with *macrophylla* giving H_{50} = type 20♀ × *macrophylla*♂ and H_{51} = reciprocal thereof. The derivative parent here very closely resembles *macrophylla* throughout in flower color and shape, habit, leaf shape, etc. $15F_1H_{50}$ and $15F_1H_{51}$, two families of 50 plants each, were equivalent in every respect. The plants were very close indeed to *macrophylla,* as is also the SESSILIFOLIA parent. The only difference readily observed was some variation in the amplitude of the corolla. In H_{51}, a plant with a larger and one with a smaller corolla were selected for pure seed. In F_2, grown in 1916, one family of H_{50} and two families of H_{51}, of 50 plants each were grown. The flower color in the F_2 populations was throughout that of *macrophylla* and the leaf characters also were those of *macrophylla.* All three families were remarkably uniform, not only agreeing with one another but uniform as to indivduals. They all resembled closely the *macrophylla* type and there was no definite segregation of any kind in them. The three populations appeared to be replicas of *macrophylla* throughout except that they were slightly more robust.

Latifolia × *angustifolia.* F_5 latifolia of type 6 was crossed with *angustifolia* giving H_{52} = type 6♀ × *angustifolia*♂ and H_{53}, its reciprocal. The derivative parent possessed the short winged petiole characteristic of latifolia of type 6. In F_1 50 plants of each cross were grown. They exhibited the long naked petiole characteristic of *angustifolia.*

In F_2 two populations of 50 plants each were grown. In color of flowers the two populations were light pink throughout, closely corresponding in this respect to *angustifolia.* In leaf shape the segregation was sharply into two classes: the stenophylla type of leaf base (long, naked petiole) and the latifolia type (shorter, winged petiole). There was some variation in the stenophylla class suggesting intermediacy between *angustifolia* and latifolia, but the forms exhibiting it showed a graded series from strict stenophylla to intermediate. The latifolia class was very uniform and sharply set off from the other class. The segregation ratios observed were as follows:

	Stenophylla	Latifolia
$16F_2H_{52}P_{16}$	42	8
$16F_2H_{53}P_{35}$	36	14
Totals	78	22

Auriculata × *macrophylla.* F_5 auriculata of type 6a was crossed with *macrophylla* giving H_{54} and H_{58} = F_5 type 6a♀ × *macrophylla*♂ and H_{55} and H_{59}, their reciprocals. It should be observed that type 6a is an early segregant from the latifolia of type 6 of H_{52} and H_{53}. In F_1 50 plants were grown of each of the four parents. All four populations were equivalent in every respect. All the plants had pink flowers, although one plant had flowers of a lighter shade than the others, and leaves of a shape somewhat intermediate between the two parents, i.e., they were more contracted at the base than *macrophylla,* but much less so than those of type 6a. One plant of H_{55}, namely $15F_1H_{55}P_{16}$, showed larger corollas than any of the other F_1 plants of any family, and was selected for further breeding.

In F_2 four families were raised and they proved to be equivalent in all respects, except as noted. There was sharp segregation for leaf shape into the sessilifolia and the sharply constricted auriculata type. In the sessilifolia class there were a number of obvious intermediates, as might be expected from the characters exhibited by F_1, but they formed a continuous series with the strict sessilifolia forms. The auriculata class did not intergrade with the dominant class.

Segregation for flower color was studied by the method described above. The color distinction between red and pink was sharp and easily drawn. In the pinks, however, there was a continuous series of shades from the deep rose pink characteristic of F_1 to the light pink typical for *angustifolia*. Numerical data are given in table 5.

TABLE 5

F_2 SEGREGATION OF PINK AURICULATA × RED SESSILIFOLIA.

Garden Numbers	Pink sessilifolia	Red sessilifolia	Pink auriculata	Red auriculata
$16F_2H_{54}P_7$	32	6	9	3
$16F_2H_{55}P_{16}$	23	11	11	5
$16F_2H_{55}P_{29}$	26	13	7	4
$16F_2H_{58}P_{26}$	26	11	7	2
$16F_2H_{59a}P_{35}$	26	12	6	2
Totals	133	53	40	16
Expected	136	45	45	15

In 1918 in connection with flower size studies three more populations of F_2H_{55} were grown. Leaves were collected from each plant and pressed, and leaf shape studies were made on these preserved specimens. The studies were not so satisfactory as those made in the field, where it is possible to examine all the leaves on a given plant; nevertheless, the data derived from the studies agreed substantially with those obtained in 1916 from field studies. It was noted in these studies that there was a distinct class of "attenuated" leaves similar to those which have been described in previous populations. Both attenuation and constriction were observed to occur in the leaves of some individuals, and this gave rise to some difficulty in classification. Numerical data are given in table 6.

TABLE 6

F_2 SEGREGATION OF SESSILIFOLIA × AURICULATA.

Garden Numbers	Sessilifolia	Auriculata
$18F_2H_{55}P_{40}$	67	28
$18F_2H_{55}P_{41}$	79	18
$18F_2H_{55}P_{46}$	79	17
Totals	225	63

Stenophylla × *angustifolia.* Reciprocal crosses were made between F_5, stenophylla of type 21 and *angustifolia;* $H_{56} = F_5$ type 21♀ × *angustifolia*♂ and H_{57}, its reciprocal. Stenophylla of type 21 has been described previously as a stable derivative closely approximating *angustifolia* in all its characters. F_1 families of 50 plants of each hybrid were raised in 1915. They were uniform throughout and so close to *angustifolia* in all characters as to be indistinguishable from it. One plant seemed to be of a slightly darker pink corolla color. $15F_2H_{56a}P_{11}$ was the only F_2 family raised. The flower color of this population was about the shade of *angustifolia* and uniform throughout the population. The family showed only a slight variation in the base of the blade such as is also seen in populations of *angustifolia.*

8. DISCUSSION OF RESULTS OF THE ANGUSTIFOLIA–MACROPHYLLA SERIES OF INVESTIGATIONS

Obviously the outstanding result of this series of investigations of hybrids between *angustifolia* and *macrophylla* is a demonstration of the complexity of the germinal differences which exist between the two varieties with respect to practically every character contrast which may be made between them. Only in one instance, the contrast between the light pink flower color of *angustifolia* and the red of *macrophylla,* is a simple Mendelian formulation possible. Here evidently the main flower color difference is dependent upon a simple allelomorphic contrast. Red × light pink gives F_1 intermediate pink, and F_2 1 red : 2 intermediate pink : 1 light pink. The red segregants breed true for red, the light pinks for light pink, and pink continues to segregate in the typical mono-hybrid fashion. Inasmuch as the intermediate pinks and light pinks form an intergrading series, it is convenient to look upon red as the recessive color. Accordingly we give this pair of factors the designation, **Rr,** following the mnemonic system advocated by Morgan, and shall so refer to it in what follows. The difficulty among the pinks appears to be due not only to phenotypic variation but also to the existence of modifying factors which have a relatively slight effect upon flower color expression. These less striking modifications of flower color we are seeking to analyze further.

In the leaf shape investigations, the complexity of the results is plainly evident from an examination of the data presented in the foregoing pages. Although the behavior here is complex, in every feature it parallels the Mendelian expectation for complex factor relations.

In F_2 the variety of leaf shapes encountered was nothing short of bewildering and series could be built up from them showing complete intergradation from one type to another. Selection of phenotypes from F_2, however, gave F_3 populations in which the complexity of segregation was usually reduced in a very definite fashion. Most of the populations exhibited fewer classes than F_2, and the selection of F_2 phenotypes held the expression of F_3 within very definite limits. Thus selection of SESSILIFOLIA forms gave in F_3 either all SESSILIFOLIA or approximately 3 SESSILIFOLIA : 1 AURICULATA. In no case did such selections give F_3 populations with STENOPHYLLA or LATIFOLIA leaf types. A summary in detail of the type of populations produced is as follows.

STENOPHYLLA selections may segregate in a variety of ways. Thus, type 1 showed approximate segregation into 3 STENOPHYLLA : 1 SESSILIFOLIA. Type 21 bred true to the STENOPHYLLA characters. Among STENOPHYLLA selections grown in 1917, population $17F_3H_2P_{17}P_6$, showed approximate segregation into 3 STENOPHYLLA : 1 AURICULATA; $17F_3H_2P_{17}P_8$, 3 STENOPHYLLA :1 SESSILIFOLIA; $17F_3H_2P_{17}P_9$ bred true for STENOPHYLLA, and $17F_3H_2P_{17}P_{22}$ gave a rather indefinite segregation of approximately 3 STENOPHYLLA : 1 SESSILIFOLIA. LATIFOLIA derivatives crossed with *angustifolia* gave F_1 STENOPHYLLA and F_2 approximately 3 STENOPHYLLA : 1 LATIFOLIA.

LATIFOLIA selections also segregate in perplexing fashion. The F_1 population of *angustifolia* × *macrophylla* is typically LATIFOLIA in its characters. LATIFOLIA under certain conditions therefore is a very complex hybrid expression. Recurrence of complex segregation of a LATIFOLIA selection is shown in F_3 of type 2. F_3 of type 3 exhibited a rather anomalous segregation ratio of petioled and sessile forms. Type 5 apparently bred true, although there was one anomalous plant in the population. Type 6 exhibited complex segregation, with an indication of a ratio of 3 LATIFOLIA : 1 AURICULATA; with subsequent establishment of both LATIFOLIA and AURICULATA in constant races. Type 7 bred true for a type of leaf like F_1; and type 9 gave approximate segregation of 3 LATIFOLIA : 1 SESSILIFOLIA.

LORIIFOLIA and LANCEOLATA derivatives are really variations of the SESSILIFOLIA type. They were both produced in constant races. Their genetic relation to the other forms is, however, not well established by this series of investigations. Although these two are really quantitative variations from the strict SESSILIFOLIA type, nevertheless, certain of our data indicate discontinuous inheritance of these contrasts.

The same quantitative factors that differentiate the narrow-leaved forms of SESSILIFOLIA from the typical broad-leaved forms may apparently differentiate narrow-leaved STENOPHYLLA, LATIFOLIA, and AURICULATA forms from the more typical broad-leaved ones. It is of interest in this connection to note that LORIIFOLIA derivatives have much narrower leaves than either of the original parents.

We have been especially interested in these LORIIFOLIA derivatives because they are somewhat like the narrow-leaved forms that Hasselbring (1912) found among Cuban tobaccos, and which are so well recognized among Cuban growers as to have received the specific designation of *lengua de vaca* or "cow's tongue." Our results indicate that it is possible for such forms to arise by segregation from crosses between broader leaved forms. The *lengua de vaca* of the Cuban growers is, therefore, probably a segregation product which could easily be eliminated by the adoption of proper pure line methods of breeding.

AURICULATA forms appear to breed true whenever segregated. The exception is type 8, which requires further investigation. It may be a leaf type similar to AURICULATA but of different genetic constitution. AURICULATA of type 10 bred true in F_3. The AURICULATA form 6a, which segregated from type 6 bred true thereafter. AURICULATA crossed with *macrophylla*, H_{54}, H_{55}, H_{58}, and H_{59}, gave SESSILIFOLIA in F_1 and in F_2 3 SESSILIFOLIA : 1 AURICULATA.

SESSILIFOLIA forms have broad sessile leaves, the distinguishing feature being merely their sessile mode of attachment. Of such selections from the original F_2 populations, four, with the exception of one anomalous plant, bred true for SESSILIFOLIA. Each of the other three populations segregated into SESSILIFOLIA and AURICULATA in about the ratio of 3 SESSILIFOLIA : 1 AURICULATA. Two SESSILIFOLIA selections were grown in 1917. One of these bred true to SESSILIFOLIA; the other gave 3 SESSILIFOLIA : 1 AURICULATA. The behavior of SESSILIFOLIA in relation to STENOPHYLLA and LATIFOLIA is explained above.

On the basis of these results we may distinguish certain definite allelomorphic pairs of factors as follows

Ss, STENOPHYLLA versus SESSILIFOLIA: **SS** being long petioled like *angustifolia*, and **ss** broadly sessile like *macrophylla*. The heterozygote may possibly approach an intermediate condition similar to LATIFOLIA.

Ll, STENOPHYLLA versus LATIFOLIA: **LL** being long petioled like *angustifolia*, and **ll** short petioled like LATIFOLIA and with a distinct but not broad wing. The contrast is really one of **SSLL**, STENOPHYLLA

versus **SSll**, LATIFOLIA. Both **ssLL** and **ssll** are probably typical SESSILIFOLIA forms. Here again the heterozygote probably shows an indistinct type of intermediacy.

Aa, SESSILIFOLIA versus AURICULATA: **AA** having the broad clasping leaf base characteristic of *macrophylla*, and **aa** the deeply constricted leaf bases with flaring auricles characteristic of AURICULATA. The contrast here is really one of **ssAA**, SESSILIFOLIA versus **ssaa**, AURICULATA, for these factors are evidently latent when in combination with **SS** or **Ss**.

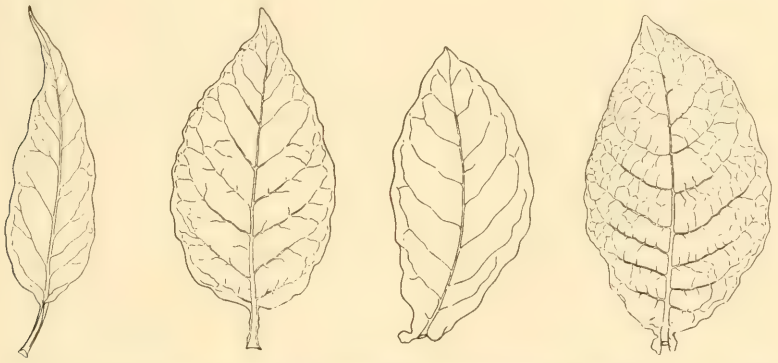

Fig. 2. Leaf base types of the *angustifolia-macrophylla* series. Left to right: STENOPHYLLA, LATIFOLIA, SESSILIFOLIA, and AURICULATA.

Some of the possible genotypes, their phenotypic expression, and genetic behavior are included in table 7. Here only monohybrid segregation is considered because it is doubtful, on account of the various types of intermediacy shown by heterozygotes, whether it would be possible to classify dihybrid and trihybrid populations satisfactorily.

TABLE 7

GENETIC BEHAVIOR OF VARIOUS LEAF TYPE GENOTYPES.

Genotype	Phenotype	Genetic behavior
SSLLAA	STENOPHYLLA	Breeds true
SsLLAA	STENOPHYLLA	3 STENOPHYLLA : 1 SESSILIFOLIA
SSLlAA	STENOPHYLLA	3 STENOPHYLLA : 1 LATIFOLIA
SSLLAa	STENOPHYLLA	Breeds true
SsLLaa	STENOPHYLLA	3 STENOPHYLLA : 1 AURICULATA
SSllAA	LATIFOLIA	Breeds true
SsllAA	LATIFOLIA	3 LATIFOLIA : 1 SESSILIFOLIA
Ssllaa	LATIFOLIA	3 LATIFOLIA : 1 AURICULATA
ssLLAA	SESSILIFOLIA	Breeds true
ssllAA	SESSILIFOLIA	Breeds true
ssllAa	SESSILIFOLIA	3 SESSILIFOLIA : 1 AURICULATA
ssllaa	AURICULATA	Breeds true

Although intermediacy of the heterozygote appears to be the normal thing in these leaf shape contrasts, it is proper to state that this intermediacy may depend to some extent upon the effect of modifying factors rather than upon the heterozygous conditon of a pair of allelomorphs. Thus the intermediate conditon between STENOPHYLLA and AURICULATA is met with in populations which do not contain AURICULATA segregation products. There are so many modifying factors in this series of investigations that it is probably impossible for us to declare definitely that in any one instance our observed segregation was wholly the result of segregation of one pair of allelomorphs. Further investigations are in progress, the purpose of which is to isolate and evaluate, if possible, certain of these subsidiary factors. For the present we can only state our certain knowledge of their existence, and our belief as to their various effects.

IV. CALYCINA–VIRGINICA SERIES

The *calycina-virginica* series of hybrids and derivatives has received much less attention than has been given to the previous series; partly because the differences between the parents are less striking and the diversity of segregation products was not so great. Two hybridizations were made: H_{18} which had *calycina* for the female and *virginica* for the male parent and H_{20} which was the reciprocal cross.

1. PARENTS OF THE CALYCINA–VIRGINICA SERIES

Elsewhere Setchell has given descriptions of *calycina* and *virginica* ("Maryland"). Like *angustifolia* and *macrophylla,* these two varieties possess distinct sets of characters which set them apart from the other *Tabacum* varieties that have been grown in the University of California Botanical Garden.

Calycina is represented in our cultures by a variety, U. C. B. G. 110/05, which was originally received from the Botanical Gardens of Cambridge University. The figure previously published (cf. Setchell, *loc. cit.,* pl. 4) well represents the general habit and type of the plant. The particular features of the characteristic teratological flower of *calycina* are better shown in plate 79, in which the leaf shape is also illustrated in more characteristic fashion. For illustrations of some of the variations which occur in the expression of the split hose-in-hose flowers the reader is referred to Goodspeed and Clausen, 1917, plate 45. The legends to the two figures of this plate should be reversed as indicated in the references to the plate in the text of this earlier article.

In stature, as previously mentioned, *calycina* belongs to the low corymbose group of *Tabacum* forms. In height the central axis usually varies between 60 and 75 cm. The laterals, however, which develop later from the base, overtop the central axis and reach a height of 120 to 135 cm. Like *macrophylla,* central axis and laterals bear close panicles of corymbose racemes, the laterals developing successively from the base. The stems and branches are stouter than those of *angustifolia* and the leaves do not droop so considerably. In these respects *calycina* occupies an intermediate position between *angustifolia* and *macrophylla.*

The leaves of *calycina,* as plate 79 will show, are sessile, but they are distinctly different from those of either *angustifolia* or *macrophylla.* Curiously enough, however, they do rather closely approximate certain of the derivatives of the *angustifolia-macrophylla* series, as, for example, the LANCEOLATA and LORIIFOLIA leaves of types 13 and 14 respectively, illustrated in plates 75 and 76. The leaves vary from broadly to narrowly lanceolate, tapering toward both base and apex, and usually with a long curved tip. The broader leaves are borne at the base of the plant, those above them becoming successively narrower in a continuous series until the linear leaves or straplike bracts of the inflorescence are reached. There are no auricles at the base of the leaf.

The inflorescence is in the form of a very close panicle of racemes, the secondary axes of which are mostly patent, and more or less recurved or bent back. The flower as a whole is of a very characteristic split hose-in-hose type. The corolla is usually split on one side, sometimes twice split, and more or less curved. The characteristic splitting of the corolla is seen even in very young buds and often the pistil protrudes from them. Typically the calyx has an elongated whitish green tube, with 3 to 5 of its tips more or less petaloid. Sometimes strips of petaloid tissue extend down the entire length of the calyx. The pod is ovoid oblong in shape. As it enlarges it splits the calyx, which then withers and drops off like the corolla, leaving a naked, whitish green capsule. The flower color is red fading to bluish purple, apparently the same as that of *macrophylla.*

Virginica is represented by U. C. B. G. 78/05, a strain received from the United States Department of Agriculture under the identification number "205–20–7." It is figured by Setchell, plate 3, and by Goodspeed and Clausen, plate 41, figure 1. The typical leaf and flower characters are well represented in plate 80 herewith.

In stature *virginica* belongs to the moderate pyramidal group of *Tabacum* varieties. It is conspicuously taller than *calycina,* the cen-

tral axis reaching a height of 150 to 175 cm. While strong laterals develop they do not originate at the base of the plant as in *calycina,* and they do not overtop the central axis. These characteristics together with the broad spreading basal leaves give the plant its pyramidal or conical shape. The leaves are very close in general shape to those of *calycina,* but they taper less abruptly to either end. The apex is prolonged into a fairly long point curved to one side, and the base is expanded into two broad, partially clasping auricles.

The inflorescence consists of a more ample panicle than that of *calycina.* The flowers are light pink in color, identical in this respect with those of *angustifolia.* The tube and infundibulum are narrow, gradually increasing in diameter from below in a funnel-shaped fashion. The corolla lobes are broad at the base, but have long, slender incurved points. Capsule and calyx present no very characteristic features, although the calyx is persistent in contrast to the deciduous calyx of *calycina.*

It will be seen from the foregoing descriptions that there are a number of distinct character contrasts between *calycina* and *virginica,* a brief note of which may well be made at this point. In flower color, red of *calycina* is contrasted with light pink of *virginica,* the same contrast which existed in the *angustifolia-macrophylla* series. Similarly the split hose-in-hose flower of *calycina* is contrasted with the normal one of *virginica;* low stature with tall; and a less decided contrast in leaf shape exists, depending upon the presence or absence of auricles at the base of the leaf.

2. F_1 of the calycina–virginica series

In the season of 1910, 55 plants of $10F_1H_{18}$ and 58 of $10F_1H_{20}$ were grown. In the season of 1911, 10 plants were grown of each of $11F_1H_{18}$ and $11F_1H_{20}$.

Like other hybrids which have been grown, these populations were uniform and equivalent throughout. It was thought that $10F_1H_{20}$ showed a more distinctly pronounced trace of *calycina* characters than did $10F_1H_{18}$, but the populations of the same seed grown in 1911 showed no appreciable difference in this respect.

In general appearance the F_1 plants resembled *virginica* more than they did *calycina.* The plants were somewhat shorter than *virginica,* running up to 135 cm. In these plants it was noted that some of the laterals overtopped the central axis as they do in *calycina.* The inflorescence was in general of the ample type characteristic of *calycina.*

The flower color was a deep pink intermediate between the two parents. The flower shape was normal throughout save that on every plant there was a small percentage of calyces with one or more pink and somewhat broadened calyx tips, or with a streak of white on one side. Sometimes these partially petaloid calyces were partly deciduous. In shape of flower the hybrid closely resembled *virginica* except that the corolla lobes were longer and more decidedly mucronate. Calyx and capsule were almost identical with those of *virginica,* the calyx being typically persistent and accrescent. The leaves were somewhat broader proportionately than those of *virginica,* but they possessed the pronounced auricles of that parent. The usual gradation in leaf shape on each plant from the broad basal leaves to the linear bracts of the inflorescence was in evidence.

The main features of H_{18} and H_{20} are well illustrated in the drawings of $10F_1H_{18}P_5$ shown in plate 81. The general habit and characters are illustrated in the photograph of $10F_1H_{18}P_{54}$ which is reproduced in plate 83, figure 1.

3. F_2 OF THE CALYCINA–VIRGINICA SERIES

In the season of 1911 four F_2 families of the *calycina-virginica* series were grown, viz., $11F_2H_{18}P_{25}$, $11F_2H_{18}P_{49}$, $11F_2H_{20}P_7$, and $11F_2H_{20}P_{26}$.

As in the *angustifolia-macrophylla* series, the segregation exhibited in those four families, comprising 97 plants, was nothing short of bewildering, and in most cases an intergrading series of forms connected one character expression with another. However, an attempt was made to classify the plants into categories suggested by the four pairs of character contrasts existing between the parents. The results of this classification are given in table 8.

TABLE 8

NUMERICAL DATA FROM F_2 POPULATIONS OF THE *CALYCINA-VIRGINICA* SERIES.

Garden Numbers	Corolla color			Corolla shape			Stature			Leaf width	
	red	pink	light pink	hose-in-hose	partial hose-in-hose	normal	short	medium	tall	broad	narrow
$11F_2H_{18}P_{25}$	6	11	6	6	8	9	17	2	5	17	7
$11F_2H_{18}P_{49}$	7	14	4	12	4	9	19	3	3	14	11
$11F_2H_{20}P_7$	5	9	11	7	8	10	11	6	8	21	4
$11F_2H_{20}P_{26}$	5	10	8	10	6	7	12	5	6	15	8
Totals	23	44	29	35	26	35	59	16	22	67	30

In this cross, corolla color behaved in exactly the same manner as it did in the *angustifolia-macrophylla* series. The same remarks as to sharpness of segregation apply here as in that series. Red was nearly always readily distinguishable, but pink and light pink formed a more or less completely intergrading series. Taking the results in this way, we obtain 23 red; 73 pink and light pink, which is substantially in accord with Mendelian expectations.

TABLE 9

F_2 SEGREGATION IN *CALYCINA-VIRGINICA* SERIES.

Garden numbers	Pink normal	Pink hose-in-hose	Red normal	Red hose-in-hose
$16F_2H_{18}P_{25}$	24	14	8	3
$16F_2H_{18}P_{49}$	25	8	14	3
$16F_2H_{20}P_7$	29	6	10	4
$16F_2H_{20}P_{38}$	30	8	11	1
Totals	108	36	43	11
Expected	112	37	37	12

With respect to corolla form some difficulty was encountered because the expression of the hose-in-hose character in the segregants did not seem to be so extreme as it was in the parent, and a large number of the plants showed slight traces of it, but sometimes in a more pronounced form than in the F_1 hybrids. Accordingly the classification of corolla form in table 8 is not a wholly satisfactory one.

The classification for stature and leaf width is subject to similar remarks as to its definiteness. Here there was also a more or less completely intergrading series of forms and no accurate measurements were taken. However, there is no doubt that there was segregation with respect to these characters, and a range of forms was obtained which completely bridged the gap between the parents. The behavior of these characters is to be considered in the light of their segregation in subsequent generations.

In 1916 four additional F_2 populations of the *calycina-virginica* series were grown in order to reëxamine populations for the segregation of normal versus hose-in-hose flowers, and red versus pink flower color. The method of classifying the flowers was that used in studies of 1916 populations previously mentioned. The results of these studies are given in table 9.

In the segregation the same grading as before of the pinks into two intergrading classes in the proportion of approximately 2 intermediate pink : 1 light pink was observed, but it was even more difficult to draw a line between light pink and intermediate pink because of the effect of the hose-in-hose conditon on flower color expression in those plants which bore teratological flowers. In the matter of segregation into normal and hose-in-hose flowers, some difficulty was experienced because some otherwise normal flowering plants bore some flowers which showed a tendency for the calyx to become petaloid, and others bore flowers which showed a very slight hose-in-hose tendency. A correspondingly slight hose-in-hose tendency is also present in F_1 plants. These plants were classified as normal. Here again it can be seen that the segregation ratios of 144 pink : 54 red and 151 normal : 47 hose-in-hose are in substantial agreement with Mendelian expectation for contrasts in a single pair of allelomorphs in each case. Moreover, the dihybrid ratio is substantially in agreement with that expected for independent segregation of the members of these two pairs of allelomorphs.

4. F_3 AND SUBSEQUENT GENERATIONS OF THE CALYCINA–VIRGINICA SERIES

In 1912 twelve F_3 families of H_{18} and five of H_{20} were grown. They will be grouped for consideration according to the characters which

TABLE 10

F_3 BEHAVIOR OF RED SEGREGANTS.

Garden numbers	Red
$12F_3H_{18}P_{25}P_{11}$	25
$12F_3H_{18}P_{25}P_{19}$	25
$12F_3H_{18}P_{49}P_{12}$	25
$12F_3H_{18}P_{49}P_{24}$	25
$12F_3H_{20}P_{26}P_{25}$	25

the F_2 parent exhibited. In table 10 the data with respect to the behavior of F_3 populations from red flowering F_2 plants are collected. Five such populations gave nothing but red flowering plants, indicating clearly that red segregants breed true. In table 11 the data from pink flowering plants are similarly collected. The reader will not fail to notice that some pink flowering selections were not heterozygous for

red. This bears out our statements as to the difficulty of classifying pink and light pink. In the seven populations which produced red flowering plants 38 plants had red flowers and 134 pink or pinkish flowers; again in substantial agreement with the behavior of flower color in the *angustifolia-macrophylla* series. The behavior of segregants classified as light pink is shown in table 12. Of the four populations from which data were gathered only one bred true to light

TABLE 11

F_3 BEHAVIOR OF PINK SEGREGANTS.

Garden numbers	Red	Pink	Light pink
$12F_3H_{18}P_{25}P_7$	5	16	4
$12F_3H_{18}P_{25}P_{21}$	....	21	4
$12F_3H_{18}P_{25}P_{25}$	6	19	....
$12F_3H_{18}P_{49}P_9$	4	20	....
$12F_3H_{18}P_{49}P_{10}$	11	13	....
$12F_3H_{18}P_{49}P_{25}$	4	16	5
$12F_3H_{20}P_{26}P_6$	4	20	....
$12F_3H_{20}P_{26}P_{11}$	4	21	....
Totals for segregating populations	38	134	

TABLE 12

F_3 BEHAVIOR OF LIGHT PINK SEGREGANTS.

Garden numbers	Red	Pink	Light pink
$12F_3H_{18}P_{25}P_{24}$	....	....	23
$12F_3H_{18}P_{49}P_{22}$	8	12	5
$12F_3H_{20}P_7P_9$	6	15	4
$12F_3H_{20}P_{26}P_5$	....	25	....

pink, one of the others bred true for pink, possibly a slightly darker shade than true light pink, and two segregated for all three colors; they must therefore have been pink heterozygotes.

In F_4 two populations each of H_{18} and of H_{20} were grown. Population $13F_4H_{18}P_{25}P_{11}P_9$ from an F_3 population breeding true for red gave in F_4 100 plants all red flowering. Population $13F_4H_{18}P_{25}P_{11}P_{13}$ from the same F_3 population gave 97 plants all red flowering like *calycina*. Population $13F_4H_{20}P_{26}P_5P_8$, which bred true for pink in F_3, gave in F_4 96 plants, all pink flowering. These three populations were grown to F_7 without showing further evident segregation. The

pink of the pink flowering derivative was at first considered somewhat darker in shade than the light pink of *virginica,* but this line also showed the hose-in-hose flower character, which sometimes makes it difficult to determine flower color accurately. In later generations of this line its color was noted as equivalent to the light pink of *virginica.*

TABLE 13

F_3 BEHAVIOR OF HOSE-IN-HOSE SEGREGANTS.

Garden numbers	Hose-in-hose
$12F_3H_{18}P_{25}P_{21}$	25
*$12F_3H_{18}P_{25}P_{24}$	23
$12F_3H_{18}P_{49}P_9$	24
*$12F_3H_{18}P_{49}P_{12}$	25
$12F_3H_{18}P_{49}P_{24}$	25
$12F_3H_{20}P_{26}P_6$	24
$12F_3H_{20}P_{26}P_{11}$	25

* Apparently not so extreme as *calycina.*

TABLE 14

F_3 BEHAVIOR OF NORMAL SEGREGANTS.

Garden numbers	Hose-in-hose	Partial	Normal
$12F_3H_{18}P_{25}P_7$	9	....	16
$12F_3H_{18}P_{25}P_{11}$	4	4	17
$12F_3H_{18}P_{25}P_{19}$	5	20	....
$12F_3H_{18}P_{25}P_{25}$	3	....	22
*$12F_3H_{18}P_{49}P_{10}$	....	4	20
$12F_3H_{18}P_{49}P_{22}$	....	....	25
$12F_3H_{18}P_{49}P_{25}$	4	21	....
$12F_3H_{20}P_7P_9$	5	20	....
$12F_3H_{20}P_{26}P_5$	7	3	15
$12F_3H_{20}P_{26}P_{25}$	10	....	15
Totals	47	153	

Taking up corolla form next, we may deal with the different populations in the same manner as was done in the case of flower color. F_3 populations from F_2 hose-in-hose segregants are recorded in table 13. Seven populations were grown, all of which bred true to the hose-in-hose character, although curiously enough two populations, $12F_3H_{18}P_{25}P_{24}$ and $12F_3H_{18}P_{49}P_{12}$ did not appear to exhibit so extreme character expressions as *calycina.*

Only one partially hose-in-hose plant was grown in F_3. For the sake of economy of space it is included in table 14, where it is marked

with an asterisk. Strangely enough, it was one of the two in the table which did not throw hose-in-hose flowers. The other normal selections all threw hose-in-hose flowering plants in the proportion of about 3 normal to 1 hose-in-hose.

In subsequent generations only the three families which were previously considered under flower color were grown. Normal flower selections from $12F_3H_{18}P_{25}P_{11}$ gave two populations, one of 100 and one of 97 plants. The plants all bore normal flowers. In $13F_4H_{18}P_{25}P_{11}P_{13}$ it was noted that some flowers were split, but there was not even a suggestion of approach to the true hose-in-hose condition. The other population $13F_4H_{20}P_{26}P_5P_8$ was from a hose-in-hose selection in the

TABLE 15

F_3 BEHAVIOR OF TALL SEGREGANTS.

Garden numbers	Tall	Short
$12F_3H_{18}P_{25}P_7$	19	6
$12F_3H_{18}P_{25}P_{24}$	1	24
$12F_3H_{20}P_7P_9$	6	19

TABLE 16

F_3 BEHAVIOR OF MEDIUM SEGREGANTS.

Garden numbers	Tall	Short
$12F_3H_{18}P_{49}P_{12}$	....	23
$12F_3H_{18}P_{49}P_{22}$	1	24

corresponding F_3 population. Ninety-four plants were grown to maturity, all of which were strictly hose-in-hose. In subsequent generations these three populations bred true to type save for the sporadic appearance of hose-in-hose flowers on plants which otherwise bore nothing but normal flowers. This, however, is not an unusual phenomenon even in pure line cultures of normal flowering varieties of *Tabacum*, and it is extremely doubtful whether the hybrid derivation of these plants had anything to do with the production of occasional split flowers.

As respects height of plants the F_3 data are given in tables 15, 16, and 17, which give the behavior of tall, medium, and short F_2 segregants respectively. The behavior here is not very convincing. Probably the difficulty in judging the character and the influence of variation in soil condition had something to do with it.

In the subsequent generations the behavior was, however, more definite. $13F_4H_{18}P_{25}P_{11}P_9$ was grown from a tall F_3 plant. No definite notes were taken as to height, but the population was noted as varying. In F_5 and subsequent generations the line bred true to tall. $13F_4H_{18}P_{25}P_{11}P_{13}$ was grown from a short F_3 plant. The ninety-seven plants were all of low stature and in subsequent generations the line bred true for low stature. $13F_4H_{20}P_{26}P_5P_8$ was grown from a tall F_3 plant. Ninety-four plants, although variable in height, all belonged in the tall class and in subsequent generations the line bred true for tall stature. Nothing but a careful biometrical study under

TABLE 17

F_3 BEHAVIOR OF SHORT SEGREGANTS.

Garden numbers	Tall	Short
$12F_3H_{18}P_{25}P_{11}$	10	15
$12F_3H_{18}P_{25}P_{19}$	16	9
$12F_3H_{18}P_{25}P_{21}$	....	25
$12F_3H_{18}P_{25}P_{25}$	5	20
$12F_3H_{18}P_{49}P_9$	1	24
$12F_3H_{18}P_{49}P_{10}$	....	24
$12F_3H_{18}P_{49}P_{24}$	7	18
$12F_3H_{18}P_{49}P_{25}$	9	16
$12F_3H_{20}P_{26}P_5$	8	16
$12F_3H_{20}P_{26}P_6$	5	19
$12F_3H_{20}P_{26}P_{11}$	....	25
Totals	68	129

well controlled cultural conditions, however, would yield results capable of strict Mendelian analysis. However, it can be said that none of the results here recorded preclude the possibility of such an analysis, although it evidently can not be done in any simple qualitative manner.

As respects leaf width it was found impossible to make even such a rough classification as was attempted in the case of stature. Here again nothing short of a strict biometrical analysis would furnish the basis for a Mendelian formulation.

As has been indicated above, three separate lines of this series were carried out to the seventh hybrid generation. Of these, one was a recombination of characters from both parents exhibiting the tall stature and normal flower of *virginica* with the red flower color of *calycina*. One exhibited a stature intermediate between that of *calycina* and *virginica* in combination with the normal flower shape of

virginica and the red flower color of *calycina*. The third had the tall stature of *virginica* and red flower color, in association with the hose-in-hose flower form of *calycina*. These three lines apparently bred true for all their characters.

5. DISCUSSION OF RESULTS OF THE CALYCINA–VIRGINICA SERIES

No extended discussion of results is indicated in connection with the *calycina-virginica* series of hybrids because particular attention was given to so few characters. Just as in the case of *angustifolia-macrophylla*, so in this series of hybrids the character differences proved to depend upon complex genotypic differences. Apparently the flower color contrast in these two varieties was the same as that in the *angustifolia-macrophylla* series, and the same relations with respect to dominance and segregation were found to hold for it. Without doubt we are dealing here with the **Rr** pair of allelomorphs as in the previous instance. The demonstration of the simple factor relations in the inheritance of the split hose-in-hose form of flower adds to our series another pair of allelomorphs which we may call **Cc** (calycine). In this case the dominance of normal over split hose-in-hose appears to be nearly, if not quite, complete. The sporadic appearance of split hose-in-hose flowers on otherwise normal plants does not even seem to be clearly associated with the heterozygous genotype, **Cc**. The data for height are not of sufficient accuracy or extent to warrant an attempt at Mendelian formulation. It was again found possible very easily to shuffle and recombine the characters occurring in the parent varieties and to establish recombination derivatives in pure lines.

V. ALBA–MACROPHYLLA SERIES

1. PARENTS OF THE ALBA–MACROPHYLLA SERIES

Alba, which is one of the parents of the *alba-macrophylla* series, is the "White" tobacco, U. C. B. G. 30/06, described by Setchell. It is one of the taller forms of *Tabacum*, ranging in height from 165 to 220 cm. Typically *alba* is unbranched below; above, it has flowering branches corymbosely arranged in succession from above downward. The leaves are sessile, more ample, more rugose, and more velvety than those of *macrophylla*. They are narrowed suddenly above the expanded, somewhat auricled and partially clasping base. The leaves

resemble those of *macrophylla* in shape but differ from them particularly in the basal portion. The corollas are white with a yellowish tinge; but in shape, size, and general proportions they are very similar to those of *macrophylla*. Line drawings of typical features of *alba* are reproduced in plate 82. The reproductions of photographs of the leaf of *alba* and of the F_1 hybrid of the *alba-macrophylla* series are shown in plate 84.

Macrophylla, U. C. B. G. 22/07, has been described above.

In these two varieties there are definite character contrasts in color of flowers, *macrophylla* being red and *alba* white; and in stature, *macrophylla* being low of stature and *alba* distinctly taller. Other contrasts also exist, although they are not so definite, in the style of branching and in the shape and texture of the leaves. Like those which have been considered above, this is a hybrid series in which the contrasts between the parent forms are of a distinctly complex character.

2. F_1 OF THE ALBA–MACROPHYLLA SERIES

The crosses between *alba* and *macropyhlla* were made in July, 1909. The cross was successful in both directions, and seed was secured from *alba*♀ × *macrophylla*♂, which was given the number H_{23}, and from the reciprocal which was given the number H_{24}.

When mature the F_1 plants were tall, 100 to 200 cm., averaging 130 to 160 cm. Habit and leaf shape were in general those of *alba*. The corolla was deep pink of about the same shade as that of the F_1 of the *angustifolia-macrophylla* series. The variation in height in these populations possibly indicates a lack of constancy in the *alba* parent in this respect. In plate 83, figure 2, is shown an F_1 plant of $10F_1H_{24}$.

3. F_2 OF THE ALBA–MACROPHYLLA SERIES

In 1911 four F_2 populations were grown, viz., 25 plants each of $11F_2H_{23}P_{13}$, $11F_2H_{23}P_{31}$, and $11F_2H_{24}P_6$, and 23 plants of $11F_2H_{24}P_{34}$. The four populations, although small, proved to be equivalent in every respect. The type of segregation was very complex. That of differences in types of leaves, especially, presented such a series of intergradations as to defy any definite classification. Likewise in height, there was a continuous series of forms from the tallest to the shortest. A rough classification was, however, made for purposes of reference into tall, medium, and short. An excellent illustration of the segregation for this character is shown in plate 85, figure 1, which shows

two adjacent plants of $11F_2H_{24}P_{34}$, one tall and of the general habit of *alba*, and the other short and of the general habit of *macrophylla*. The classification for height is given in table 18. Obviously no satisfactory Mendelian formulation can be deduced from these data.

As regards flower color, however, the classification is more definite. Four more or less distinct shades were distinguishable, viz., red, pink, light pink, and white. The pink and light pink shades merged into each other, consequently they have not been separately recorded in table 18. Bearing in mind the previous behavior of red and pink, as shown in the *angustifolia-macrophylla* and *calycina-virginica* series,

TABLE 18

F_2 SEGREGATION IN THE *ALBA-MACROPHYLLA* SERIES.

Garden numbers	Stature			Flower Color		
	tall	medium	short	red	pink	white
$11F_2H_{23}P_{13}$	11	6	8	3	14	8
$11F_2H_{23}P_{31}$	13	6	6	4	12	9
$11F_2H_{24}P_6$	7	10	8	3	15	7
$11F_2H_{24}P_{34}$	6	8	9	6	13	3
Totals	37	39	31	16	54	27

it would appear that we are here dealing with dihybrid populations in which a pair of allelomorphs for color versus white is concerned in addition to that pair upon which the contrast of pink versus red was found to depend. The pair of allelomorphs for the pink versus red contrast has been represented by **R** and **r**, respectively. If we represent the contrast of color versus white by **W** and **w**, respectively, the two parents in this series would possess the following genotypes:

Alba = **RRww**
Macrophylla = **rrWW**

The light pinks of the previous series would then be **RRWW**, and the factor **R** might be regarded as a dominant diluter. According to this formulation, F_1 of the *alba-macrophylla* series would be **RrWw**, pink, and F_2 should segregate in the ratio 3 red : 9 pink : 4 white. The expected result in the classification of ninety-seven plants in whole numbers is 18 red : 55 pink : 24 white. Agreement is thus fairly close.

A check on the results above noted for the 1911 sowings of the F_2 population was made by growing in 1916, five additional F_2 popula-

tions of the same series, viz., $16F_2H_{23}P_5$; $16F_2H_{23}P_{32}$; $16F_2H_{23}P_{34}$; $16F_2H_{24}P_{28}$; and $16F_2H_{24}P_{33}$. The segregation in the resulting populations is recorded in table 19.

The method of studying these flowers was the more accurate one previously described in connection with later generations of the *angustifolia-macrophylla* series. In the classification of flowers it was noted that reds and whites were sharply distinguishable from pinks. The pinks were of many different shades; some very light, others relatively dark, corresponding to the range obtained in the *angustifolia-macrophylla* series. However, in these populations the range of variation of pink appeared to be greater and the intergradations more gradual than in that series. In the whites there was also evidence of differentiation into classes depending upon the amount of yellow or creaminess in the flowers. Some of the whites appeared to belong to a clear white albino class, but most of them had a distinctly creamy tinge. The observed segregation in these populations was in almost exact agreement with the formulation advanced above.

TABLE 19

F_2 SEGREGATION IN 1916 SOWINGS OF *ALBA-MACROPHYLLA SERIES.*

Garden numbers	Red	Pink	White	Totals
$16F_2H_{23}P_5$	11	18	11	40
$16F_2H_{23}P_{32}$	12	19	11	42
$16F_2H_{23}P_{34}$	8	24	9	41
$16F_2H_{24}P_{28}$	5	34	11	50
$16F_2H_{24}P_{33}$	7	29	14	50
Observed	43	124	56	223
Expected	42	125	56	223

4. F_3 AND SUBSEQUENT GENERATIONS OF THE ALBA–MACROPHYLLA SERIES

In table 20 we have summarized the behavior of the F_3 populations as respects color of flowers and height of plant. Of the five red F_2 plants from which F_3 populations were grown, three proved to be homozygous for red and one proved to be a heterozygote of the genetic constitution **rrWw**. Of this latter selection two sowings were made, one in 1912 and another in 1913. The combined results from these two sowings, 35 red: 14 white, are in fair agreement with Mendelian

expectation. The other population exhibited an anomalous type of segregation, and gave 2 red : 23 white. It is unfortunate that this line was not investigated further, but the results probably are due to an experimental error.

TABLE 20

F_3 SOWINGS OF THE *ALBA-MACROPHYLLA* SERIES.

F_2 Phenotypes		Garden Numbers	Flower Color			Stature	
Flower Color	Stature		Red	pink	white	short	tall (or medium)
Red	Tall (or M)	$12F_3H_{23}P_{13}P_3$	19	....	6	25	....
	Short	$12F_3H_{23}P_{31}P_1$	22	.	..	22	....
	Sh't (or M)	$12F_3H_{23}P_{31}P_{17}$	25	...	....	4	21
	Tall	$12F_3H_{23}P_{31}P_{22}$	24	..	..	4	20
	Tall	$12F_3H_{23}P_{31}P_{25}$	2	...	23	1	24
	Tall	$16F_3H_{23}P_{13}P_3$	16	...	8	....	....
Pink	Tall	$12F_3H_{23}P_{13}P_{13}$	2	14	6	8	10
	Medium	$12F_3H_{23}P_{13}P_{25}$	6	12	7	22	..
	Tall	$12F_3H_{23}P_{31}P_7$	6	14	5	..	25
	Tall	$12F_3H_{23}P_{31}P_{19}$	10	9	4	...	23
	Medium	$12F_3H_{23}P_{31}P_{20}$	6	12	5	4	19
	Tall	$12F_3H_{24}P_6P_5$	3	22	..	..	25
	Short	$12F_3H_{24}P_{31}P_{18}$	5	13	7	24	1
	Tall	$12F_3H_{24}P_{34}P_{20}$		20	5	..	25
	Tall	$16F_3H_{24}P_{34}P_{20}$		13	4	...	...
White	Tall	$12F_3H_{23}P_{13}P_{14}$	.	...	25	....	25
	Tall	$12F_3H_{23}P_{13}P_{15}$	.	.	25	....	25
	Short	$12F_3H_{23}P_{13}P_{24}$	..	..	25	25	..
	Tall	$12F_3H_{24}P_6P_2$	..	..	24	....	24
	Short	$12F_3H_{24}P_6P_3$	....		25	4	21
	Tall	$12F_3H_{24}P_6P_4$	..	.	23	..	23
	Short	$12F_3H_{24}P_{34}P_{23}$		1	23	25	..
	Short	$13F_3H_{23}P_{13}P_{24}$		.	10	10	.
	Short	$13F_3H_{24}P_{34}P_{23}$	...		10	10	..

Eight families of F_3 plants were grown from pink F_2's. Of these F_2 plants six proved to belong to the **RrWw** genotype. The totals from these six populations, viz., 35 red : 74 pink : 34 white, are in fair agreement with the dihybrid ratio 3 red : 9 pink : 4 white. One of the other populations gave 3 red : 22 pink. It was probably the result of sowing seed from an F_2 plant of the genetic constitution **RrWW** which should give 3 pink : 1 red. The observed segregation ratio is not good, but the numbers are small. Two sowings of $F_3H_{24}P_{34}P_{20}$ gave totals of 33 pink : 9 white. The F_2 plants in this case must have been of the genetic constitution **RRWw**; in which case expectation would be 3 pink : 1 white. No selection was observed to breed true

for pink in F_2. This, however, is not inexplicable, for only one in nine among the F_2 pinks should belong to the **RRWW** genotype.

Sowings were made from seven white F_2 plants. Among 190 plants so produced there was one pink flowering individual. It surely represents some kind of experimental error. We may say, therefore, that for flower color the formulation advanced to account for the F_2 segregation ratio, also accounts for the behavior observed in the various F_3 populations.

We have reported the data on height in table 20, largely in order to show that this character, although obviously dependent on factor differences, is so complex as not to permit of a simple qualitative treatment. Thirteen F_3 sowings from tall F_2 plants gave ten populations showing only tall plants. Two of the remaining populations showed segregation into 31 tall (and medium) : 13 short. One population consisted entirely of short plants. The classification of the F_2 parent of this plant as "tall" was noted as doubtful at the time, the note "or medium" being appended. Two populations were grown from F_2 plants of medium height. One of these populations was uniformly of low stature; the other showed segregation into 19 tall (and medium) : 4 short. Six populations were grown from F_2 parents, four of which apparently bred true for low stature, the other two showed segregation into tall (and medium) or short in the ratio 42 : 8. It is interesting to note that at the time of classification the parents of these two later populations were classified as short (or medium), indicating a doubt as to proper classification. More definite data will be necessary before a satisfactory formulation of these height differences can be made, but certain of our results seem to indicate that there is one allelomorphic pair which has a rather marked effect on stature, and that there are other subsidiary pairs of factors which have less marked effects.

Only one line in this *alba-macrophylla* series was carried out to subsequent generations to demonstrate the possibility of fixing character complexes from a hybrid. It was a low stature white flowering line. In F_4, 100 plants of $13F_4H_{24}P_{34}P_{23}P_2$ bred true to low stature and white flower color. The population was uniform, the plants exhibited the general habit of *macrophylla* rather than that of *alba;* and the leaves were the same shape as those of *macrophylla*, but they were slightly rugose, although not so much so as those of *alba*. In F_5, two populations of 25 plants each were grown, viz., $14F_5H_{24}P_{34}P_{23}P_2P_{47}$ and $14F_5H_{24}P_{34}P_{23}P_2P_{83}$. No differences were detectable between these two populations, and the characters exhibited were those we have noted

for F_4. In F_6 10 plants each of $15F_6H_{24}P_{34}P_{23}P_2P_{47}P_5$ and $15F_6H_{24}P_{34}P_{23}P_2P_{83}P_{12}$ and in F_7 10 plants each of $16F_7H_{24}P_{34}P_{23}P_2P_{83}P_{12}P_8$ and $16F_7H_{24}P_{34}P_{23}P_2P_{47}P_5P_8$ were grown. In both cases the parallel populations were equivalent and the characters exhibited and described in F_4 remained constant. Plate 85, figure 2, is a good illustration of the type of this family as fixed. A photograph of the original F_2 plant, from which the family descended, is reproduced in plate 85, figure 1. It will be noted that the derivative represents a fixation of the characters of the original F_2 selection, and that no important segregation occurred in it either in F_3 or in subsequent generations.

5. DISCUSSION OF RESULTS OF THE ALBA–MACROPHYLLA SERIES

Here again, as in the *calycina-virginica* series, no extended discussion of results is necessary. Obviously the differences separating the two varieties are of a complex nature genetically as in the two previous cases. The series demonstrates the existence of another pair of allelomorphs for flower color in this group of *Tabacum* varieties, viz., **Ww**, and the part played by it in the production of both red and light pink flower color has been determined. The height contrast again proves to be too complex for qualitative Mendelian formulation. As in the previous cases, the establishment of stable recombination derivatives proved to be a simple task.

VI. GENERAL CONCLUSIONS

We shall limit the discussion of these results to three main topics upon which these investigations seem to have thrown some light: (1) the origin and interrelationships of varieties of *Tabacum;* (2) the methodology of Mendelian analysis in *Tabacum;* and (3) Mendelian heredity in *Tabacum.*

1. ORIGIN AND INTERRELATIONSHIPS OF VARIETIES OF TABACUM

As a result of extensive studies of a considerable assemblage of *Tabacum* varieties, Comes (1905) came to the conclusion that the species *Tabacum* could be subdivided into six fundamental varieties:

a. var. *fructicosa* Hook.
b. var. *lancifolia* (W.) Comes.
c. var. *virginica* (Agdh.) Comes.
d. var. *brasiliensis* Comes.
e. var. *havanensis* (Lag.) Comes.
f. var. *macrophylla* Shrank.

Inasmuch as practically every *Tabacum* variety shows combinations of characters of two or more of these fundamental varieties, Comes assumed them to have been derived mostly through hybridization between the fundamental varieties, and he proceeded from purely morphological studies to classify the different commercial varieties on the basis of their supposed hybrid derivation. Anastasia (1906), who has criticized this scheme of Comes very severely, reduced the number of fundamental varieties to four, striking out *fructicosa* and *lancifolia* from Comes' list, and substituting *purpurea* for *macrophylla*. Although disagreeing as to the fundamental varieties, Comes and Anastasia seem to agree in referring existing varieties to derivation, mostly through hybridization, from a relatively small number of fundamental varieties.

The Howards (1912) object to the mode of classification of Comes and Anastasia, and point out as a result of their studies of types of Indian tobaccos that no attempt at classification based on derivation can be considered seriously unless supported by actual experimental studies. In her later paper in particular Miss Howard (1913) shows that segregation products may be obtained through hybridization which transcend the limits set by the parents. The Howards propose a scheme of classification based primarily upon leaf and habit characters, and they adopted this morphological system purely as a provisional means for facilitating identification and reference among the numerous forms of Indian tobaccos.

Our results agree with those stated by the Howards, and we raise the same objection to schemes of classification such as Comes and Anastasia have advocated. Any scheme of classification based on morphological considerations alone cannot well meet with the approval of geneticists, for it does not take into account genotypic differences which exist among forms of similar morphological appearance. Thus it is possible, as Miss Howard points out, by crossing different members of a given group to obtain segregation products which belong in an entirely different morphological group in the scheme of classification. In particular she points out that "petiolate" forms have been produced as segregation products from two "sessile" parents, yet "petiolate" and "sessile" have been used as primary indexes for classification of tobaccos into groups.

The difficulty from the genetic point of view with any classification of *Tabacum* varieties is the same as that which is met with in the classification of varieties of other polymorphic species. Taking the species as a whole and viewing the entire assemblage of its varieties,

there is evidently in *Tabacum,* to those who accept current interpretations of heredity, a series of allelomorphic contrasts, the number of which cannot even be guessed, but which need not perhaps be more numerous or striking than those which have been discovered in *Drosophila.* But whereas in *Drosophila* the factors have been kept in stocks involving for the most part single factor differences from a common wild type, in *Tabacum,* and in other cultivated crop plants such as barley, maize, oats, rice, wheat, etc., these factor differences have been shuffled about through long periods of cultivation until existing varieties are no longer related clearly to a common form or to each other. In some instances in such groups certain factor differences have a more striking visible effect than in others. In such instances we have an obvious mode of classification based not upon number of factor differences so much as upon the striking character differences which arise from certain factor contrasts. Thus in barley we have the classification of varieties advocated by Harlan (1918) based upon recognition of a number of major morphological distinctions, some of which at least have been clearly analyzed in Mendelian fashion; and the same principle has been recognized in the classification of varieties of maize, where it has led to the absurdity of erection of a heterozygous form, podded maize (*vide* Collins), as one of the primary group distinctions. In some instances, doubtless, the sorting of factors may give rise to certain recombinations which are more favorable to life processes than others, as Muller has pointed out in another connection, and such genotypes may act as centers around which groups of varieties may be built up, thus giving rise to more or less obvious grouping of varieties. The attempt to base a system of classification upon reference to certain fundamental types does not, however, promise much simplification of the difficulty; moreover, such an attempt rests upon the rather naive assumption that it is unnecessary to account for the fundamental types.

From a genetic standpoint, therefore, it would appear that in attacking the problem of classification and interrelations of varieties in a polymorphic species the major premise should be a recognition of the fundamental equivalence of every homozygous genotype. Starting from this premise a system of dichotomy beginning with those factor contrasts which produce the most striking, visible effects and proceeding to those of lesser effect might be set in operation. Such a system obviously would in certain cases separate some similar varieties into separate groups, and would lead to recognition of group differences without obvious morphological distinctions, but the system

would have a real significance, and the relationships indicated by it would be fundamental ones. It is, however, necessary to have a much more extensive knowledge of Mendelian heredity in *Tabacum* than we have at present before such a system can be formulated.

2. METHODOLOGY OF MENDELIAN ANALYSIS IN TABACUM

From the Mendelian side there are certain obvious facts associated with *Tabacum* as a species. In the first place, as we have stated before, the species is highly polymorphic. A large and striking assemblage of varieties exists, the most extreme of which hybridize readily and give fully fertile hybrids and full fertility in their derivatives. A few teratological forms are known in which fertility is somewhat reduced, but the above generalization does not far overstate the facts. The species is, moreover, so highly polymorphic that with respect to any given character a representative collection of varieties may be arranged in a series connecting the most extreme expressions of that character by imperceptible steps. Thus in flower color we have represented in the collection of varieties of the University of California Botanical Garden dark red, red, light pink, pinkish, and white, and descriptions occur in the literature which indicate the existence of further shades of red connecting these. Now flower color is a rather definite character, comparatively speaking, for it appears to be little affected by ordinary environmental conditions. In many polymorphic forms, such for example as maize, there are a large number of such definite characters, and as a consequence studies of inheritance in these forms have resulted in definite Mendelian analysis of many character differences. But in *Tabacum* unfortunately most of the characters involve quantitative elements, and these with few exceptions depend so largely for their particular expression upon environmental conditions that it becomes a difficult matter in a segregating population to distinguish between those differences which are inherent and referable to the genotype and those which have come about through the action of extrinsic forces. And yet our assemblage of tobacco varieties indicates clearly that there are genotypes which give rise to all possible expressions in these characters. Here we find the reason for the present backward state of knowledge of inheritance in *Tabacum*, for while there have been numerous investigations which indicate clearly that the Mendelian mode of transmission may be followed in all these character differences, yet there are very few investigations which have resulted in the precise type of factor analysis characteristic

of investigations with other forms, specific mention of which is unnecessary.

The general features of inheritance in *Tabacum* varietal crosses are plain enough. The results of our investigations in this connection agree throughout with the conclusions which Miss Howard drew from her studies. When we are dealing with complex differences, the F_1 is commonly intermediate in character expression between the two parents. Not only is this true as respects the F_1 plant as a whole but it is also true for individual characters. The F_2 commonly consists of a varied assemblage of forms covering the range between the two parents, or even not uncommonly presenting products not included in the range between the two parents. So many and of such variety are the forms obtained that accurate classification is entirely out of the question. But in F_3 and in subsequent generations segregation, even for characters commonly regarded as quantitative, sometimes occurs in distinct discontinuous classes in marked contrast to the intergrading series of forms obtained in F_2. This is shown particularly well in our analysis of leaf base factors, for in this case we have been able to adopt a qualitative mode of attack on one of the features which contributes to leaf shape. If such an analysis proves successful in one instance, there seems to be little reason why it should not be extended to others. There is, therefore, additional evidence in this successful application of the mode of qualitative analysis to quantitative characters in support of the oft repeated contentions of East (1913), Hayes (1912), Hayes, East, and Beinhart (1913), Miss Howard (1913), and others that fundamentally the same mode of inheritance holds for quantitative characters in tobacco as for qualitative ones. The distinction between the two classes of characters is purely an artificial one erected for the purpose of convenience in formal treatment, and at most depending merely upon an increase in complexity of the factor relations involved and on the greater fluctuation of the characters in response to environmental differences.

The question remains to be discussed whether semiquantitative characters admit of a qualitative mode of analysis, and if so, how? Miss Howard (1913) as a result of her extensive studies of inheritance in Indian tobaccos concludes that the easiest way to determine the principles underlying inheritance in these forms is to establish as many extracted homozygous intermediate forms as possible. The establishment of such forms in themselves, however, is only a step in the Mendelian analysis of the differences. Such forms are, as might have been expected on theoretical grounds alone, less different from one

another and from the parents than the original parents are from each other. Moreover, our experiments show that as a result of simplification of the factor differences the derivative strains crossed with each other or with the parents give F_2 progenies which often exhibit clear-cut segregation in characters which showed intergrading series in the original F_2 population. In other populations, however, from crosses between derivatives, the populations still exhibit perplexing complexities which make classification difficult and uncertain. In such cases we could again resort to the method of establishing intermediate derivatives from them; but if the number of factors concerned in a given character is even moderately large, as is certainly the case with many of these quantitative characters, the number of genotypically different derivatives which may be secured becomes so great as to make the method impracticable.

Our experience indicates that the successful factor analysis of these quantitative character differences depends not only upon getting what Castle (1919) has called the residual heredity equivalent throughout the population, but also in establishing the proper kind of residuum which will most emphasize the character differences associated with the pair of factors or pairs of factors under investigation. The problem may be illustrated crudely by considering the pair of flower color factors **Rr**. If the residuum should contain **PP**, the effect of which is described below, segregation would give **PPRR**, **PPRr**, and **PPrr**. In character expressions these three different genotypes would doubtless all be of various shades of dark red, difficult or impossible of accurate separation. With such a residuum, therefore, it would be impossible to investigate satisfactorily inheritance in the factor pair **Rr**. But if we should substitute **pp** for **PP** in the residuum, the segregation products would be **ppRR** and **ppRr**, which would be pink, and **pprr**, which would be red. Here the segregation would be sharp and distinct, and there would be practically no difficulty in classification. How complex such interrelations can be has been shown most clearly by Bridges (1919) in his account of specific modifiers of eosin in *Drosophila*. As Bridges shows it would easily be possible to obtain populations of *Drosophila* defying classification, but by keeping the factors separate and studying their character effects with known residual genotypes, it has been possible to determine and locate the factors involved. Doubtless much of the extraordinary success of Mendelian analysis in *Drosophila* has been due to the fact that factor differences arose under conditions such that the residual genotype gave no difficulty; whereas in crop plants, the geneticist starts with

long established diverse types, evidently related to one another in fundamentally the same manner as are the various *Drosophila* mutants, but more complexly, and from these complex assemblages he must unravel the tangled skein of heredity.

There are, however, other and perhaps quicker ways of establishing a uniform and favorable residual heredity than that of securing and testing homozygous extractives, and these may be employed in certain special cases. Thus, if it be desired to study the relationship of the pair of factors **Ss** for the petioled versus sessile condition, it should be possible to proceed by crossing back the F_1 of *angustifolia* × *macrophylla*, for example, to *macrophylla*, selecting the petioled forms from the back cross for again crossing back to *macrophylla*, and continuing the process until clear-cut segregation was obtained. Such a mode of procedure should establish a residual genotype equivalent to that of *macrophylla* itself, and should thereby enable the student eventually to study the effect of substituting **SS** for ss in the *macrophylla* genotype. In tobaccos technical details make it particularly easy to adopt such a procedure, but it is useless to speculate further upon its results until it shall have been attempted.

3. MENDELIAN HEREDITY IN TABACUM

From the standpoint of factor analysis, we have demonstrated clearly in the foregoing pages, the existence of a number of distinct pairs of factors. Two of these affect flower color, one flower form, and three affect the character of the leaf base. The particular effects of the opposing members of these pairs of factors and the interrelations which they exhibit so far as these have been investigated have been set forth in the discussions which follow the description of each of the three series of hybrids. Although evidently many other factor differences were concerned in these studies, and remain for further investigation, the results which we have described make a beginning toward a more accurate knowledge of Mendelian heredity in *Tabacum*.

So far as our results furnish any data on the question, the six pairs of factors isolated exhibit no linkage relations. The data here are far from complete, but the results are in accordance with theory. According to White (1912), there are twenty-four pairs of chromosomes in *Nicotiana*. Assuming for the sake of discussion that each of these pairs of chromosomes bears a set of factors comparable in numbers to any other pair, then the chances of finding linkage when only six pairs of factors are studied is very slight. This large number

of pairs of chromosomes may account for the ease with which recombination pure lines were established. Even with a large number of factor differences, such as evidently distinguish these *Tabacum* varieties, the chances are slight with so many pairs of chromosomes that linkage will enter in as a factor to cause the continued preservation of a heterozygous condition as a consequence of selection for a certain set of characters.

It remains to consider those portions of the *Nicotiana* literature which deal specifically with the Mendelian inheritance of the characters which we have investigated, and to harmonize our results with those which have been reported previously. Unfortunately there have not been many investigations in *Tabacum* which have been prosecuted far enough to arrive at a definite factor analysis of the differences under consideration. The investigations of Miss Howard (1913), promise of the continuation of which has not thus far been fulfilled, in general confirm those which we have presented in this paper. On the strictly analytic side, however, Miss Howard did not carry her work very far. This doubtless was due to the difficulty of making a factor analysis of the characters which she selected for study, viz., (1) time of flowering, (2) height of stem, (3) arrangement of the leaves on the stem, (4) length of the decurrent portion of the lamina, (5) venation of the leaf, (6) leaf shape, and (7) undulation of the surface and margin of the leaf. For most of these characters she demonstrates, by the presentation of numerical data in some cases as far as F_4, the probability of the character differences in question depending upon multiple factor differences. In the case of height certain of her cultures strongly suggest the existence of a pair of allelomorphs, which has a relatively great effect, for in some of her cultures there are definite discontinuous height differences. For the inheritance of length of the decurrent portion of the lamina Miss Howard postulates the existence of at least three or four distinct pairs of factors. As respects leaf base, she records the synthesis of petiolate types from sessile parents, observing in two cases a simple 1:2:1 segregation into petiolate : intermediate : sessile. As respects corolla color, she records one F_2 population from pink × very pale pink fading into white which consisted of 72 pinks of various shades to 45 whites, but some of the palest pinks were indistinguishable from white. She found evidence of grouping among the pinks, and postulates the existence of two factor differences to account for it. The investigations which we have reported do not throw light upon the factor constitution of the very pale pink varieties with which Miss Howard worked. Our

varieties *angustifolia* and *virginica* have lively pink flowers. Of the paler pinks or "pinkish" forms we have a representative in our *N. Tabacum* var. Cavala, U. C. B. G. 72/05, which has flowers distinctly lighter in color than those of *angustifolia* or *virginica*. Our petiolate forms also seem to be of different constitution from those with which Miss Howard worked, for she presents evidence to show that hers are combinations of recessive factors and that they breed true whenever they occur as segregation products, whereas our petiolate forms often gave plants with sessile leaves as segregation products. We have, however, secured evidence that some distinctly short petiolate forms arise from sessile ones, perhaps by modifications of the AURICULATA leaf type in the direction of stripping the auricle and lower portion of the lamina from such leaves, but our results are not yet definite enough to permit of rigid formulation. Further investigation of the relationships of the various petiolate forms is necessary.

As respects flower color Allard (1919) has presented some interesting data which at first sight appear to contradict those which we have presented. Allard found that carmine × pink gave F_1 carmine and F_2 3 carmine : 1 pink. The back crosses gave consistent data. Thus F_1 carmine × carmine parent gave all carmine, and F_1 carmine × pink parent gave 1 carmine : 1 pink. In F_3 pink segregants bred true for pink, and carmine either bred true for carmine or gave again 3 carmine :1 pink. The difficulty here is that our red is not genetically identical with Allard's carmine. Our flowers of *macrophylla* and *calycina* at full expansion show a color lying between rose red and pomegranate purple of the Ridgway color scale. This color, which we have called red for the sake of brevity, is very close to carmine, but we have another flower color, which we call dark red, represented by *N. Tabacum* var. *macrophylla purpurea*, which is probably identical with the Giant Red flowering tobacco which Allard used in his experiments.

We have made some preliminary tests of this dark red, and find that it behaves differently from red. Crossed with our white it gives dark red in F_1, instead of pink as was obtained from red × white. Since our white carries the factor **R**, which is responsible for the production of pink flower color, dark red must differ from pink in a dominant factor. If we call this factor pair **Pp**, then our various colors of tobacco would have the following genotypes:

Dark red	**WWRRPP**
Red	**WWrrpp**
Pink	**WWRRpp**
White	**wwRRpp**

Obviously this formulation would account for Allard's results without contradicting those which we have presented, but inasmuch as our experimental evidence is not yet complete we refrain from any further discussion of the consequences of this scheme save one. Allard presents certain data for a cross of carmine × white which gave in F_1 light carmine, and in F_2 3 colored : 1 white, the colored being various shades of carmine and pink. Allard's discussion of this case is somewhat mixed, but he evidently erroneously expected a simple monohybrid segregation of the 1 : 2 : 1 kind. That more than one factor is concerned in the cross is clearly shown by the results of crossing some of the extracted whites with pink varieties. The results of three such crosses gave:

1. Pink (Maryland Mammoth) × Extracted white	36 carmine : 18 pink
2. Extracted white × Pink (Maryland Mammoth)	20 carmine : 23 pink
3. Pink (Conn. Broadleaf) × Extracted white	12 carmine : 39 pink
Totals	68 carmine : 70 pink

In (1) above we have combined in the carmine class 17 *carmine* and 19 *somewhat lighter than carmine.*

If we consider a cross of dark red × white according to the genetic formulation given above, the F_1 should be dark red, and F_2 should consist of 9 dark red : 3 pink : 4 white. Doubtless the pinks and the dark reds would exhibit various shades, but the three classes should be distinct. If we combine "carmine" and "lighter than carmine" to form a carmine class and dark and light pink to form a pink class, Allard's F_2 data reduce to the following form:

149 carmine : 64 pink : 65 white.

This ratio compares very favorably with a 9 : 3 : 4 expectation, viz.:

157 dark red : 52 pink : 69 white.

No F_3 results from sowings from colored F_2 plants are given, but the single F_2 white, which gave when crossed with pink approximately equal numbers of carmine and pink flowering plants, is accountable for as of the genotype **wwRRPp.** Further investigations are in progress for the purpose of determining precisely the relation of dark red and pinkish to the red, light pink, and white colors reported upon in this paper.

There are other references in the literature to Mendelian inheritance in *Tabacum,* but inasmuch as these do not bear upon the characters which we have attempted to analyze it does not appear necessary to discuss them at this point.

VII. SUMMARY

Studies of three intervarietal crosses in *Tabacum* demonstrate that:

1. All the differences between varieties of *Tabacum* can be analyzed in a Mendelian fashion, if sufficient refinement in methods be introduced.

2. Stable recombinations of parental characters can readily be obtained with three or four generations of self-fertilization.

3. Characters outside the range between the parents are sometimes produced following hybridization, and these may be readily established in stable lines by self-fertilization.

4. The petioled leaf base of *angustifolia* and the sessile leaf base of *macrophylla* differ in at least three pairs of factors.

5. A single factor difference exists between normal and split hose-in-hose flowers.

6. Two pairs of factors account for the relation existing between red, light pink, and white flower color. A third pair of factors is necessary to account for dark red.

On the theoretical side it has been pointed out that:

1. Derivation of relationships and erection of systems of classification after the manner of Comes and Anastasia cannot be relied upon unless supported by experimental evidence.

2. An adequate scheme of classification should be based upon identities and dissimilarities in the genotypes, irrespective of the derivation of the forms in question.

3. Mendelian analysis in *Tabacum* requires that special attention be paid to residual portions of the genotype, so that the factor differences under consideration act in a stable residuum most favorable for emphasis of the character differences under investigation.

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EXPLANATION OF PLATES

A special note is due the illustrations in this paper. The line drawings were made by Miss Anna Hamilton and Miss Helen M. Gilkey. Special attention was paid to accuracy in proportions and details. No attempt, however, was made to represent the characteristic *Nicotiana* pubescence. The photographs require no special mention save that sometimes the garden number given in the legend does not correspond with that given on the label in the photograph. The difference is due to a change in system of numbering used in F_3 and in subsequent populations. In this paper, in order to avoid confusion, garden numbers from the beginning have been made to conform to this change. The legends of all the plates have been made more complete than is usual in order to facilitate cross-reference and to enable the reader to grasp their essential significance more readily.

PLATE 55

Fig. 1. *Nicotiana Tabacum* var. *angustifolia*, U. C. B. G. 68/07. A typical plant of *angustifolia* at the height of its blooming period. The laterals overtopping the central axis and the long-petioled *stenophylla* form of leaf are especially to be noted. The drooping of the leaves is very characteristic of this variety.

Fig. 2. *Nicotiana Tabacum* var. *macrophylla*, U. C. B. G. 22/07. A typical plant of *macrophylla* at the height of its blooming period. Note especially the stout laterals overtopping the central axis and the *sessilifolia* type of leaf.

Fig. 1

Fig. 2

PLATE 56

Nicotiana Tabacum var. *angustifolia*, U. C. B. G. 68/07. Line drawings of typical details of *angustifolia*. In the upper right-hand corner the characteristic straplike sessile leaf or bract of the inflorescence. Upper left, details of bud, flower, and capsule. Lower right, details of pistil and stamens. Lower left, the typical long-petioled *stenophylla* leaf of *angustifolia*. Leaves × ½; flowers and capsules natural size.

PLATE 57

Nicotiana Tabacum var. *macrophylla*, U. C. B. G. 22/07. Line drawings of typical details of a plant of *macrophylla*, showing floral details and the extreme variations in leaf size and shape on the plant. Leaves × ½; flowers and capsules natural size.

PLATE 58

Fig. 1. *Nicotiana Tabacum* var. *angustifolia*, U. C. B. G. 68/07. Typical leaves of *angustifolia* of the *stenophylla* type showing the range of variation on a single plant.

Fig. 2. *Nicotiana Tabacum* var. *macrophylla*, U. C. B. G. 22/07. Typical leaves of *macrophylla* of the *sessilifolia* type showing the range of variation on a single plant.

Fig. 1

Fig. 2

PLATE 59

Angustifolia-macrophylla series, F_1 leaves.

Fig. 1. Typical leaf of $10F_1H_2P_{56}$, an F_1 of the *angustifolia-macrophylla* series. Note the short, winged petiole and the clasping auricles.

Fig. 2. Typical leaf of $10F_1H_3P_{58}$, a variation from the usual *latifolia* type of the F_1 leaf. Note the shorter petiole, less conspicuously winged condition, and the almost total lack of auricles.

Fig. 3. Typical leaf of $10F_1H_4P_{55}$. The petiole is somewhat longer than that normal for the F_1.

Fig. 4. Typical leaf of $10F_1H_5P_{56}$. The petiole here is shorter than that normal for the F_1.

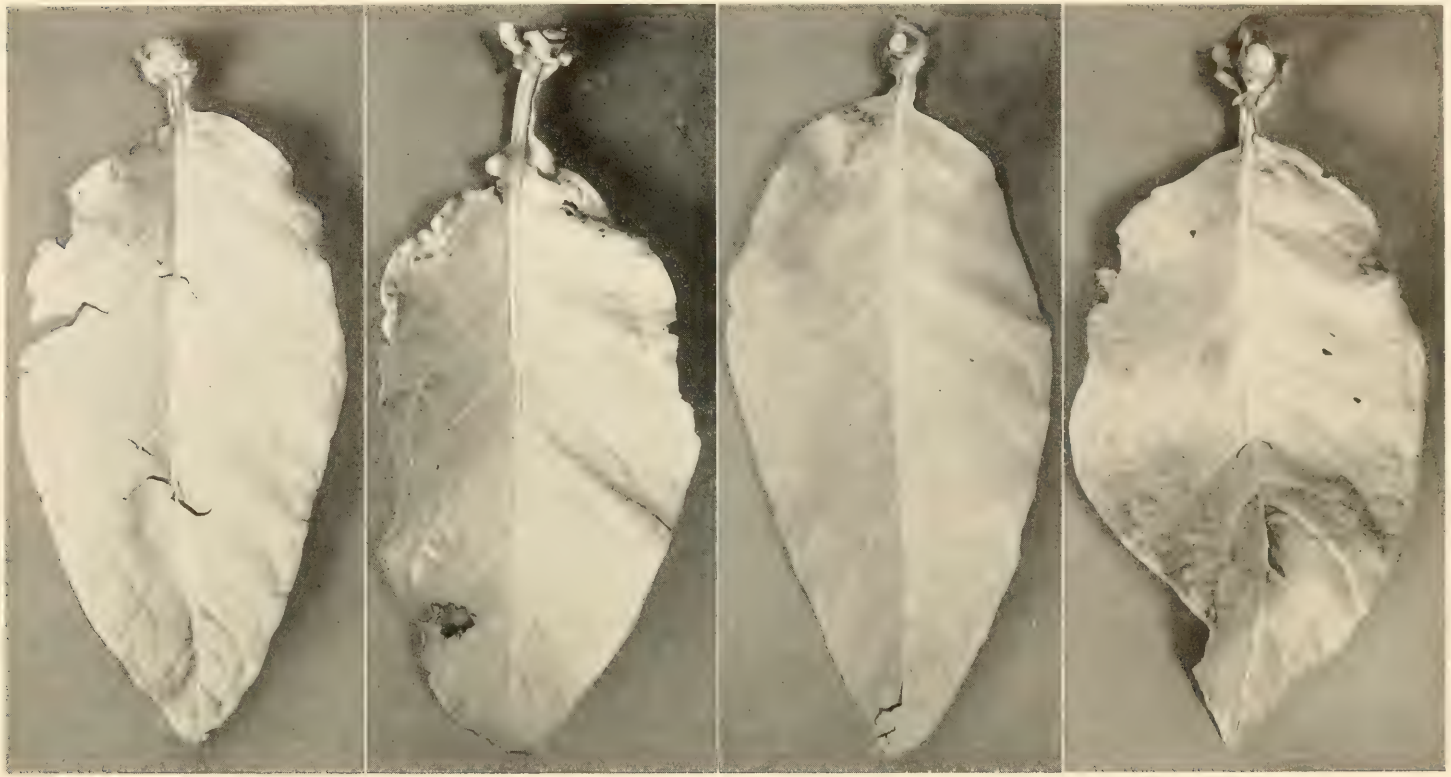

Fig. 1 Fig. 2 Fig. 3 Fig. 4

PLATE 60

Portions of inflorescences of *N. Tabacum* var. *macrophylla, N. Tabacum* var. *angustifolia* and the F_1 hybrid between them.

Fig. 1. Left, portion of inflorescence of *macrophylla,* middle, of the F_1, and right, of *angustifolia.*

Fig. 2. Left, portion of the inflorescence of *macrophylla,* middle, two of the F_1, and right, of *angustifolia.*

Fig. 1

Fig. 2

PLATE 61

Angustifolia-macrophylla series, F_1 plants.

Fig. 1. Photograph of $10F_1H_2P_{56}$, the F_1 plant from which the leaf shown in plate 59, figure 1, was taken.

Fig. 2. Photograph of $10F_1H_3P_{58}$, the F_1 plant from which the leaf shown in plate 59, figure 4, was taken.

Fig. 1

Fig. 2

PLATE 62

Angustifolia-macrophylla series, line drawings of F_1.

Line drawings showing morphological details of the typical F_1 plant of the *angustifolia-macrophylla* series. The garden number of the plant was $10F_1H_{15}P_7$. Leaves × ½; flowers and capsules natural size.

PLATE 63

Angustifolia-macrophylla series, type 1.

Line drawings of morphological details of F_2 of type 1. The garden number was $11F_2H_2P_7P_{49}$. Note particularly the *stenophylla* type of leaf. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 16 *stenophylla* of type 1 and 9 *lanceolata* of type 14.

PLATE 64

Angustifolia-macrophylla series, type 2.

Line drawings of morphological details of F_2 of type 2. The garden number was $11F_2H_2P_3P_{30}$. The leaf is of the *latifolia* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 12 *latifolia*, 8 *sessilifolia*, and 4 *auriculata*.

PLATE 65

Angustifolia-macrophylla series, type 3

Line drawings of morphological details of F_2 of type 3. The garden number was $11F_2H_2P_3P_{14}$. The leaf is of the *latifolia* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 4 *latifolia* and 10 *sessilifolia*.

PLATE 66

Angustifolia-macrophylla series, type 4.

Line drawings of the morphological details of type 4. The garden number was $11F_2H_2P_7P_{18}$. The leaf is an extreme form of the *latifolia* type. Leaves × ⅓; flowers and capsules natural size.

No progeny was grown from this plant.

PLATE 67

Angustifolia-macrophylla series, type 5.

Line drawings of morphological details of type 5. The garden number was $11F_2H_4P_{41}P_{14}$. The leaf is of the *latifolia* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 24 *latifolia* and 1 *auriculata*.

PLATE 68

Angustifolia-macrophylla series, type 6.

Line drawings of morphological details of F_2 of type 6. The garden number was $11F_2H_4P_2P_{18}$. The leaf is of the *latifolia* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 5 *stenophylla*, 17 *latifolia*, and 4 *auriculata* plants, but the segregation was not distinct.

PLATE 69

Angustifolia-macrophylla series, type 7.

Line drawings of morphological details of F_2 of type 7. The garden number was $11F_2H_2P_1P_{15}$. The leaf is of the *latifolia* type. Leaves $\times \frac{1}{3}$; flowers and capsules natural size.

The F_3 progeny of this plant was uniformly of the same type as the parent, and the line bred true in subsequent generations.

PLATE 70

Angustifolia-macrophylla series, type 8.

Line drawings of morphological details of F_2 of type 8. The garden number was $11F_2H_2P_2P_{40}$. The leaf approached the *auriculata* type. Leaves $\times$ $^1/_3$; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 16 *sessilifolia* and 8 *auriculata*, indicating that the F_2 plant was an extreme variant of the heterozygous *sessilifolia-auriculata* condition.

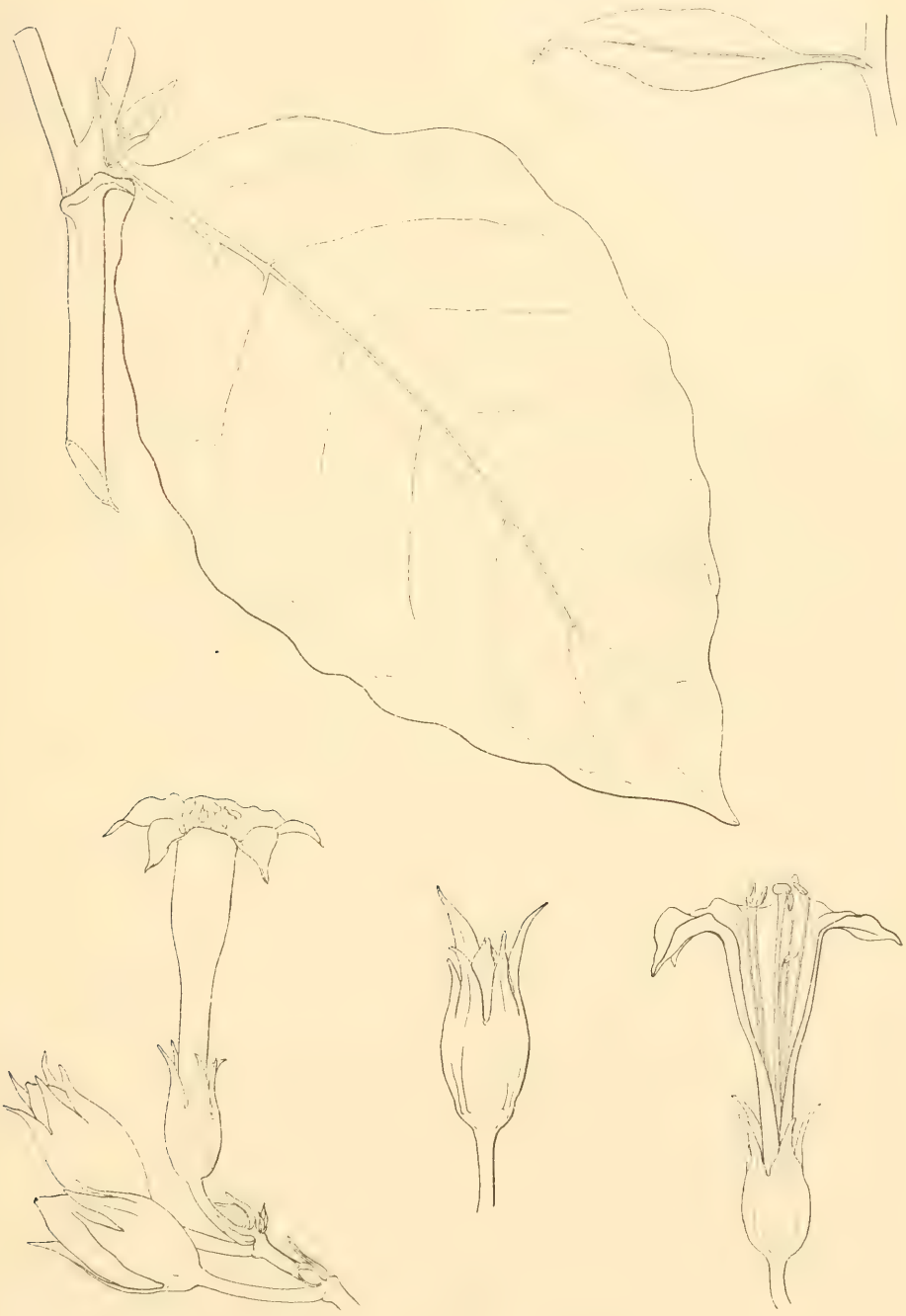

PLATE 71

Angustifolia-macrophylla series, type 9.

Line drawings of morphological details of F_2 of type 9. The garden number was $11F_2H_4P_{41}P_8$. The leaf is of the *latifolia* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 18 *latifolia* and 7 *sessilifolia*.

PLATE 72

Angustifolia-macrophylla series, type 10.

Line drawings of morphological details of F_2 of type 10. The garden number was $11F_2H_4P_{41}P_{17}$. The leaf was of the *auriculata* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted uniformly of *auriculata* plants.

PLATE 73

Angustifolia-macrophylla series, type 11.

Line drawings of morphological details of F_2 of type 11. The garden number was $11F_2H_4P_{41}P_9$. The leaf is of the *sessilifolia* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant was uniformly of the same *sessilifolia* type.

PLATE 74

Angustifolia-macrophylla series, type 12.

Line drawings of morphological details of F_2 of type 12. The garden number was $11F_2H_4P_{41}P_{12}$. The leaf is of the *loriifolia* type. Leaves $\times$ ⅓; flowers and capsules natural size.

The F_3 progeny of this plant was uniformly of the same *loriifolia* type, and two constant races, one with red and one with light pink flowers, were obtained from it.

PLATE 75

Angustifolia-macrophylla series, type 13.

Line drawings of morphological details of F_2 of type 13. The garden number was $11F_2H_2P_3P_{44}$. The leaf is of the *lanceolata* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant was uniformly of the same *lanceolata* type.

PLATE 76

Angustifolia-macrophylla series, type 14.

Line drawings of morphological details of F_2 of type 14. The garden number was $11F_2H_2P_3P_{38}$. The leaf was classified as *sessilifolia*, although strictly it is intermediate between *sessilifolia* and *lanceolata*. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 24 plants of the same *sessilifolia* type and 1 "filler," which had leaves more like *auriculata* of type 8.

PLATE 77

Angustifolia-macrophylla series, type 15.

Line drawings of morphological details of F_2 of type 15. The garden number was $11F_2H_2P_3P_{10}$. The leaf is of the *sessilifolia* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant was uniformly of the same leaf type.

PLATE 78

Angustifolia-macrophylla series, type 16.

Line drawings of morphological details of F_2 of type 16. The garden number was $11F_2H_2P_3P_{80}$. The leaf is of the *sessilifolia* type. Leaves × ⅓; flowers and capsules natural size.

The F_3 progeny of this plant consisted of 15 *sessilifolia* and 8 *auriculata*.

PLATE 79

Nicotiana Tabacum var. *calycina*, U. C. B. G. 110/05.

Line drawings of morphological details of leaf and flower of *calycina*. The leaf is of the *lanceolata* type, and the flowers are of the conspicuously teratological, split hose-in-hose form. Leaves × ½; flowers and capsules natural size.

PLATE 80

Nicotiana Tabacum var. *virginica*, U. C. B. G. 78/05.

Line drawings of morphological details of leaf and flower of *virginica*. Contrast the normal flowers and auricled leaves with the corresponding details of *calycina*, shown in plate 79. Leaves × ¼; flowers and capsules natural size.

PLATE 81

Calycina-virginica series, an F_1 plant.

Line drawings of morphological details of an F_1 plant of the *calycina-virginica* series. Note the normal flowers and the slightly auricled leaves. Leaves × ¼; flowers and capsule natural size.

PLATE 82

Nicotiana Tabacum var. *alba*, U. C. B. G. 30/06.

Line drawings of morphological details of flower and leaf of *alba*. Note especially the rugose leaf. Compare this drawing with those of *macrophylla* shown in plates 57 and 58, figure 2. Leaves × ⅓; flowers and capsule natural size.

PLATE 83

Fig. 1. *Calycina-virginica* series, an F_1 plant. A typical F_1 plant of the *calycina-virginica* series. This plant is at the height of its blooming period. The garden number was $10F_1H_{18}P_{54}$.

Fig. 2. *Alba-macrophylla* series, an F_1 plant. Photograph of a typical F_1 plant of the *alba-macrophylla* series. The garden number was $10F_1H_{24}P_{54}$. The plant is at the height of its blooming period.

Fig. 1

Fig. 2

PLATE 84

Alba-macrophylla series, F_1 leaves.

Fig. 1. Photograph of typical leaves of *alba*.

Fig. 2. Photograph of a typical leaf of $10F_1H_{24}P_{17}$, F_1 of the *alba-marcophylla* series.

Fig. 3. Photograph of a typical leaf of $10F_1H_{24}P_{34}$, F_1 of the *alba-macrophylla* series.

Compare these leaves with those of *macrophylla*, shown in plate 58, figure 2. The rugoseness of *alba* has been carried over, to a somewhat reduced extent, into the F_1 hybrid.

Fig. 1

Fig. 2

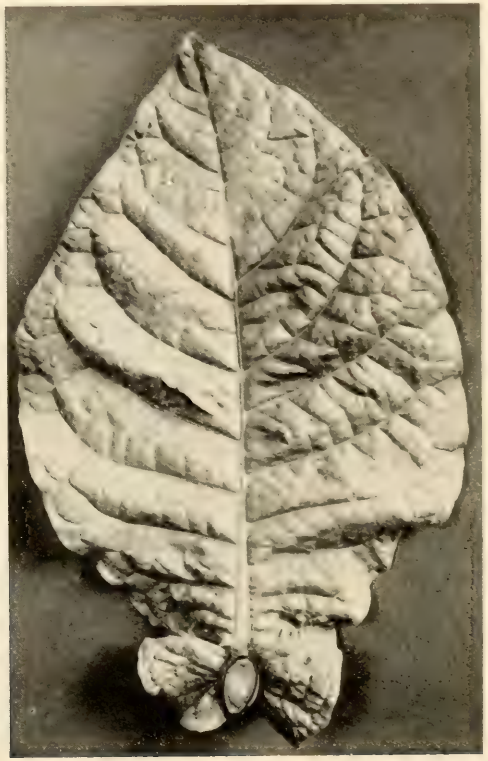

Fig. 3

PLATE 85

Fig. 1. *Alba-macrophylla* series, F_2 plants. Photograph of two adjacent plants, $11F_2H_{24}P_{34}P_{22}$ and $11F_2H_{24}P_{34}P_{23}$, from the same F_2 population of the *alba-macrophylla* series. An illustration of segregation for height in this population.

Fig. 2. *Alba-macrophylla* series, an F_4 plant. Photograph of a typical F_4 plant, $13F_4H_{24}P_{34}P_{23}P_2P_{20}$, of a dwarf line of the *alba-macrophylla* series. The line here illustrated was derived from the dwarf F_2 plant shown in figure 1. This line has bred true for seven generations.

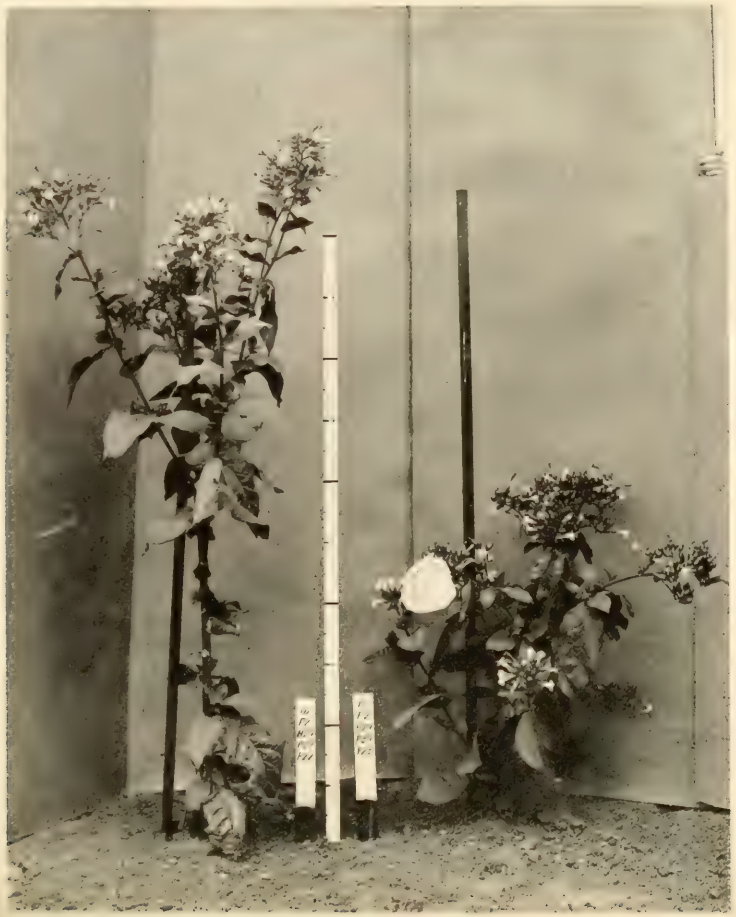

Fig. 1

Fig. 2

INDEX*

Abscise cells, definition of, 349.

Abscission, 192, 196, 274, 275, 304, 359, 367, 371, 383, 384, 403, 412, 429; relation of nutrition to, 281-289; effect of non-fertilization, 289, 348; effect of physiological condition of plant, 294, 348; automatic, 296, 359, 433; axial, 348, 351, 415; floral, 293–298, 366, 408, 413, 417, 444, 448; lateral, 348; effect of sugar, 381; relation to, of development of mechanical tissue in pedicel, 370; spontaneous, 390–396, 443; a physiological problem, 417; relation of fertilization to, 431–433; caused by premature pollination, 433.

Process of, 353–358, 375, 376, 413; time, 358, 385, 389, 414, 443; influence on, of age of flower, 396, 414, 416; of humidity, 397; moisture requirement, 448.

Induction of: factors in, 359–360, 362, 416; in *Nicotiana*, 371–373; in *Lycopersicum*, 373–374; in *Datura*, 374–375; in pedicel, 399-404, 414; *experimental:* by illuminating gas, 397–404, 414; by action of acids on separation cells, 404–406; by mechanical injury, 406–411, 414. *See also* Cell separation; *Citrus;* Corolla; Fertilization; Lamella; Pedicel; Pollination; Style, etc.

Abscission of Flowers in *Nicotiana* and *Citrus*, Effects of Illuminating Gas and its Constituents in Causing, 439.

Abscission of Flowers and Fruits in Solanaceae with Special Reference to *Nicotiana*, 347.

Aesculus, 351.

Age, of leaf, 444; of flower, 229, 396, 414, 417; of plant, 229, 240, 444; of seed, 451; influence of, in germination, 453–454, 455.

Alba-macrophylla series, 461, 504–510; description of *alba*, 504, contrasted with *macrophylla*, 505; generations of, 505, 507; tables showing segregation in hybrids, 506, 507, 508; discussion of color and height, 508, 509; results of investigation, 510.

Allard, H. A., cited, 518–519.

Allelomorphs, 483, 490, 499, 504, 506, 512, 517.

Ampelopsis, 350, 351.

Anastasia, G. E., cited, 511.

Angustifolia-macrophylla series, 461, 462–494; parents, 463; description of *angustifolia*, 463–465; description of *macrophylla*, 465-467; F_1 generation, 467–469; F_2 generation, 469–471, 483–487; F_3 and subsequent generations, 472-481, 483–487; table of F_3 families, 473; chart showing relationships of families, 474; six types of parents selected, and characterization of, 474–475; *auriculata*, 475, 477, 479, 485, 488, 489, 492, 493; *lanceolata*, 478, 491; *latifolia*, 476–478, 488, 491, 492; *loriifolia*, 478–479, 491, 492; *sessilifolia*, 480–481, 485, 487, 489, 492, 493; *stenophylla*, 475–476, 485, 490, 491, 492; summary of color observations in, 482–483; classification with respect to leaf base modifications, 486; crosses of derivatives with parents, 487-490; complexity of germinal differences, 490.

Aristolochia Sipho, 353.

Arntzen, V., acknowledgment, 435.

Asparagus, 350.

Atropa, 350.

Auriculata derivatives, 475, 479, 484-485; × *macrophylla*, 488; × *sessilifolia*, 489.

Ayres, A. H., 273.

Begonia, 350, 352.

Beinhart, E. G., cited, 514.

"Boll-shedding" in Egyptian cotton, 287.

Brazilian tobacco, 4; illustrations of, opp. 32.

Bridges, C. B., cited, 515.

Brunfelsia, 351.

Bud variations in *Nicotiana* hybrids, 459.

Calycina-virginica series, 494–504; parents, 494; description of *calycina*, 494–495; description of *virginica*, 495–496, opp. 572; F_1 generation, 496–497; F_2 generation, 497–499; corolla color, 497–501; corolla form, 501; F_3 and subsequent generations, 499–504; complex genotypic differences, 504; results of investigation, 504.

Capsule in hybrids, 468, 470.

* Univ. Calif. Publ. Bot., vol. 5.

Carbon dioxide, 447, 448, 449. *See also* Narcotic vapors.
Carbon monoxide, 449. *See also* Narcotic vapors.
Cavala tobacco, 5, 233; illustration of, opp. 34, 463.
Cell separation, with abscission, 354, 413, 416; method of, 376–384; enzyme problem of, 383; time of, in spontaneous abscission, 396; induction of, 404; figures showing, opp. 422, 424, 428.
Cell turgor, 376, 379–380, 413, 404, 413.
Cestrum fasciculatum, 366, 371, 398, 411.
Chloroform. *See* Narcotic vapors.
"Chorismus," 355.
Cinnamomum, 354.
Citrus, abscission of flowers in, 439, 441, 443; effects of illuminating gas, 445, 449. *See also* Abscission.
Citrus limonia, 445.
var. Eureka, 441, 449.
sinensis var. Washington Navel, 441, 445, 446, 449.
var. Valencia, 441, 449.
Clausen, R. E., 301, 435, 457.
Comes, cited, 2, 3, *passim*, 458, 510, 511.
Complexity of character differences in *Tabacum* varieties, 459–460, 468, 470, 491.
Controlled Pollination in *Nicotiana*, 429.
Corolla, abscission of, 357; in *Nicotiana*, 383, 413; illustrated, 383; in *Datura*, 384, 413.
Corolla color in *calycina-virginica* series, 497–501.
Corolla diameters, in *N. acuminata*, quantitative expression of imperfect dominance in, 117–168; plates showing fluctuations in, opp. 164, 166, 168; tables of measurements of, 224–226; method of measuring, 227. *See also* Dominance, imperfect.
Crocus, effect of poisonous gases on germination in, 446.
Cross-pollination, 127, 135, 189, 248, 263, 267, 269, 275, 277, 293, 295, 387.
Cuba, *lengua de vaca* tobacco, 492.
Cucumis, 351.
Cucurbita, 359.
Culture series in *Nicotiana Tabacum*. See *Alba-macrophylla; Angustifolia-macrophylla; Calycina-virginica*.
Cuphae, 350.
Cytology, study of, in relation to abscission in *Nicotiana* hybrids, 296, 432; in "separation layer," 352; in genera of the Solanaceae, 366; changes in, accompanying abscission, 376–377; of the pedicel in *Nicotiana*, 363–365; illustrated, 363; in *Lycopersicum*, 365-366; illustrated, 365.
Darkness and light, influence of, on germination, 451.
Datura, 350, 367, 382, 402, 403, 412; abscission of style and corolla, 383–385.
sanguineum, 366, 398, 411.
stramonium, 444.
Davidson, Pirie, 429.
Dominance, Law of, 174.
Dominance, in inheritance of characters in *Nicotiana* hybrids, 111, 112, 118, 312, 313, 314, 316, 320, 321.
Imperfect, in corolla diameters, 117, 168; experimental materials, 119; growing conditions, 123; description of *Nicotiana* flower, 125; technique and method, 126–132; experimental work, 132, 136; measurement of diameters, 138; results, 144, 145; literature, 152; plates showing, opp. 164, 166, 168.
Don, G., cited, 2.
Drosophila, 512.
Drought, effect on abscission, 397.
East, E. M., cited, 514.
Environment, influence on *Tabacum*, 513.
Erodium, genus, 357.
Ether. *See* Narcotic vapors.
Ethylene, 449. *See also* Narcotic vapors.
Euonymus, 354.
Exfoliation, distinction between, and abscission, 348.
Ferric chloride, used in nutrition experiment on *Nicotiana*, 285.
Fertilization, relation of, to abscission, 387, 391, 407, 416, 431–433; selective, 434.
Floral abscission, 294–298, 366, 408, 413, 417, 444, 448; following non- or unsuccessful pollination, 296; histological and cytological investigation of, 296.
Floral characters in F_1 hybrids: 457, *passim*; color, 307–311, 313; leaf, 307–311; flower (habit, size, etc.), 223–227, 307–311, 312; inheritance of, 169–174, 228, 229, 230, 435; "unit-character" problem in, 175; influence of age, 229; range of variability, 229–230; plates showing, 324–344; apparatus for measuring size, 435–438. *See also* Corolla diameters; Mendelian notation, etc.

Flower form, factor in Mendelian heredity in *Tabacum*, 516.
Flower Measurement, Apparatus for, 435; illustrations of, 436, 437.
Fort Bidwell, California, 120.
Fuchsia, 352, 359.
Gasteria, 350.
Geranium, genus, 357.
Genetic aspects of problem of heredity in *Nicotiana* hybrids, 301.
Genotypes in *Tabacum*, 513, 520; genetic behavior of leaf type of, 492–493, 516.
Germination of tobacco seed, 96–100, 108, 109, 136, 199, 233, 451; sulfuric acid method, 199–200, 203-209, 219, 220; value of separation of seed, 200; seed used, 201; germinating conditions, 202, 203; relation between age of seed and its viability, 209–215, 220, 233, 240; hybrid vs. parent seed, 215-219, 220, 241–247, 248; rate and amount of germination, 240–247, 248; response to stimulation by hybrids and parental pollen, 276-281; effect of narcotic vapors on, 446; relation of light and darkness to, 451–455; germinal differences in culture series of *Nicotiana Tabacum* hybrids, 490.
Germination of Tobacco Seed, Notes on, 199, 233, 451.
Goldschmidt, R., acknowledgment, 435.
Goodspeed, T. H., 87, 169, 189, 199, 223, 233, 249, 273, 293, 301, 429, 435, 439, 451, 457; acknowledgment, 417.
Groth, B. H. A., work of, on F_1 hybrids, 147.
Harlan, H. V., cited, 512.
Hasselbring, H., cited, 492.
Hayes, H. K., cited, 514.
Helianthemum, genus, 357.
Heredity, "unit character" conception, 174–186; physiology of, 177-181, 197, 301; use of hypothetical terms in connection with, 181; conception of reaction system contrasts in, 361; genetic aspects of problem, 301; residual, in *Nicotiana Tabacum*, 515–516, 520.
Hibiscus rosasinensis, 444.
Histological investigation of abscission in *Nicotiana* hybrids, 296.
Histology of the pedicel, 350, 412; in *Nicotiana*, 363–365; in *Lycopersicum*, 365–366; in other genera of the Solanaceae, 366.
Hodgson, R. W., 439.
Howard, Gabrielle L. C., cited, 511, 514, 517, 518.
Humidity, influence on abscission time, 397.
Hydrogen, used in abscission experiments, 447.
Illuminating gas, induction by, of abscission, 397–404, 443; relation of concentrations of, to leaf-fall, 444. *See also* Narcotic vapors.
Illuminating Gas and its Constituents, Effects of, in Causing Abscission of Flowers in *Nicotiana* **and** *Citrus*, 439; composition of, 440; methods of experiment, 440; results, 441, 442.
Illumination, effect on abscission, 397.
Impatiens Sultani, 348, 350, 352.
Inheritance in *Nicotiana* **Hybrids, Studies in,** 87, 169, 223.
Inheritance in *Nicotiana Tabacum*, **A Report on the Crossing of Certain Varieties,** 457; quantitative characters, 459, 513, 514–516; bud variations, 459; interspecific hybrids, 459; Mendelian, 459, 460 (*which also see*); occurrence of strays, 462; features of, 514; residual heredity, 515–516, 520; linkage as a factor in, 516–517; flower form factor in, 516; color, flower, leaf base, leaf shape, 457, *passim*; petiole, 457, *passim*.
Injury, mechanical, 416. *See also* Abscission.
Iochroma tuberosa, 366, 398, 399, 441.
Kendall, J. N., 293, 347, 443.
Lagenaria, 351.
Lamella, middle, dissolution of, 356, 376, 378, 379, 382, 384, 416.
Lanceolata derivatives, 474, 478, 491.
Latifolia derivatives, 474, 476–478; × *angustifolia*, 488; a complex hybrid, 491.
"Law of Dominance," 174.
"Law of Segregation," 174.
Leaf characters (base, habit, shape) in hybrids, 307, 311, 467, *passim*.
Light and darkness, influence of on germination, 451, 455.
Linkage as a factor in inheritance in *Tabacum*, 516–517.
Linum, genus, 357.
Lipman, C. B., acknowledgment to, 289.
Literature cited, 30, 152, 187, 198, 222, 231, 248, 290, 299, 322, 450, 520; summary of literature on abscission, 350–361.
Litmus, reaction of *Nicotiana* to, 381.
Lloyd, F. E., acknowledgment to, 289, 417.
Loriifolia derivatives, 474, 478–479, 491–492, 495.
Lupinus, 446.

Lycium, 367.
australis, 366, 398, 399, 411.
Lycopersicum, histology of pedicel, 350, 351, 367, 412; development of separation zone, 367–369; figure showing, 368; increase in size and development of mechanical tissues of pedicel, 369–371; spontaneous abscission in, 393, 401, 408, 414; floral abscission in, 408; figures showing abscission process, opp. 428.
Lycopersicum esculentum, spontaneous abscission in, 395.
var. *pyriforme*, 398, 399, 411.
var. *vulgare*, 398, 399, 411.
McGee, J. M., 439.
Magnesium, used in nutrition experiment on *Nicotiana*, 284.
Maryland tobacco, 5, 89; illustrations of, opp. 36, 461, 463.
Mendelian dominance, 111, 112, 118, 117–168.
Mendelian inheritance in *Nicotiana Tabacum*, 459, 460, 471, 487, 490, 503, 506, 507–508, 510, 513, 516–519, 520; methodology of Mendelian analysis in, 513–516; pairs of factors, 516; consideration of literature in connection with present study, 517–519.
Mendelian notation, 181, 183, 185, 186, 228.
Mexico, white tobacco from, 7.
Mineral acids, action of, on separation cells of *Nicotiana*, 404–406.
Mirabilis, 352, 353, 445.
Jalapa, 348, 353.
Moisture, contributing factor in germination of tobacco seed, 451.
Moisture requirement in process of abscission, 448.
Muller, H. J., cited, 512.
Narcotic vapors, effect of, on abscission, 355, 359, 390, 397–404, 416, 445, 447. *See also* Illuminating gas.
Nature of the F$_1$ Species Hybrids between *Nicotiana sylvestris* and Varieties of *Nicotiana Tabacum* with Special Reference to the Conception of Reaction System Contrasts in Heredity, 301; plates showing, opp. 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344.
"*Nic. tabaccum* Cuba," 251, 263, 264, 265, 266, 268, 269, 274, 275.
Nicotiana, literature cited, 30, 152, 187, 198, 222, 231, 248, 270, 290, 299, 322, 367, 418, 450.
Nicotiana, **Parthenogenesis, Parthenocarpy, and Phenospermy in**, 249.
Nicotiana, **Studies in**, 1.
Nicotiana acuminata, 23, 262, 350; plate showing, opp. 158, 168; description of wild form of, 121; plate showing, opp. 160; dominance experiments on corolla diameters, 117–168. *See also* Dominance, Imperfect.
grandiflora, 119, 120; plate showing, opp. 82.
parviflora, 119, 120.
acutiflora, 22.
affinis, 16, 22, 23, 261, 448.
alata, 17, 22, 262; plate showing, opp. 80.
var. *grandiflora*, 16, 22, 23.
var. *persica*, 22.
Bigelovii, 25, 121, 234, 262, 398, 399, 411.
"*Pomo.*" 398.
var. *Wallacei*, 26, 398.
Forgetiana, 23.
glauca, 18, 262, 303, 398, 411.
glutinosa, 18; plate showing, opp. 72.
Langsdorffii, 15–17, 262, 350, 392, 435; plate showing, opp. 64.
var. *grandiflora*, 16, 23, 393, 406, 407; plate showing, opp. 66.
var. *longiflora*, 17; plate showing, opp. 68.
longiflora, 21–22; plate showing, opp. 78.
var. *acutiflora*, 21.
multivalvis, 27, 234, 398, 399, 411.
noctiflora, 20; plate showing, opp. 76.
var. *albiflora*, 20.
Palmeri, 29.
paniculata L., 17, 263; plate showing, opp. 70.
plumbaginifolia, 398.
quadrivalvis, 26, 234, 387, 398, 399, 411.
repanda, 28; plate showing, opp. 84.
rustica, L., 12, 262, 350, 398, 411, 454, 455.
var. *asiatica*, 12; plate showing, opp. 48.
var. *brasilia*, 13, 399; plate showing, opp. 50.
var. *humilis*, 13; plate showing, opp. 52.
var. *jamaicensis*, 13, 14; plate showing, opp. 54.
var. *pumila?*, 15; plate showing, opp. 60, 62.
var. *scabra*, 141; plate showing, opp. 56.
var. *texana*, 13, 15, 233; plate showing, opp. 58.
Sanderae, 398, 399, 411.
suaveolens, 24, 122, 387, 398, 411.

sylvestris, 29, 189, 261, 262, 277, 296, 302, 303, 350, 387, 388, 389, 411, 448, 459; plate showing, opp. 86.
Tabacum, 3, 261, 296, 302, 350, 352, 353, 454, 455; types of, 4–11, 460–461; plate showing hybrid seedlings of, opp. 168; races in, 458–459, 491; taxonomic problems, 461; origin and interrelationships of varieties of, 510-513; influence of environment, 513; teratological forms in, 513; residual heredity in, 515-516, 520.
var. *alba*. See *Alba-macrophylla* series.
var. *angustifolia*, 7, 9, 10, 233, 262; description of (height, habit, leaf, flower, etc.), 463-465. See also *Angustifolia-macrophylla* series.
var. *auriculata*, type derivative, 475, 479, 484–485; × *macrophylla*, 488; × *sessilifolia*, 489.
var. *brasiliensis* (''Brazilian''), 4, 262, 463.
var. *calycina*, 6, 262, 461; plate showing, opp. 38.
''Cavala,'' 262, 463. *See also* Cavala tobacco.
var. *colossea*, 19.
var. *commutata*, 16.
var. *fruticosa*, 6, 7.
var. *havanensis*, 4.
var. *lancifolia*, 6.
var. *macrophylla*, 5, 8, 89, 90, 233, 262, 277, 398, 399, 411, 461–463; description of, 465–467; contrasted with *alba*, 505. See also *Alba-macrophylla*; *Angustifolia macrophylla*.
var. *purpurea*, 10–11, 189, 262, 441; plate showing, opp. 46.
''Maryland,'' 5, 233, 262, 390, 398, 401, 411, 461.
var. *sanguinea*, 11.
var. *sessilifolia*, type derivative, 475, 480, 485; × *macrophylla*, 487; × *auriculata*, 489.
var. *stenophylla*, type derivative, 474, 475–476, 484, 485; × *auriculata*, 489.
var. *virginica*, 6, 89, 90, 461.
tomentosa, 18, 19, 262, 303; plate showing, opp. 74.
trigonophylla, 28.
var. *ipomopsiflora*, 29.
var. *pulla*, 29.
var. *sordida*, 29.
vincaeflora, 24, 122.
viscosa, 23.

Nicotiana hybrids, inheritance in, 87, 117, 169, 223, 457, *passim*; weight of seeds, 87–116; technique and methods of experiment, 126–131; flowering tendency, 304.
Seed: size, 91; color and weight, 92; germination, 96–101; development, 101–103; conclusions regarding, 108, 109; abortive (phenospermic), 265. *See also* Germination of tobacco seed; Mendelian inheritance in *Nicotiana Tabacum*.
Nicotiana **Hybrids Made with** *N. sylvestris* **as a Parent, Partial Sterility of**, 189, 273, 293.
Nicotiana **Hybrids, Quantitative Studies of Inheritance in**, 87, 169, 223.
Nicotiana Tabacum, **Inheritance in. A Report on the Results of the Crossing of Certain Varieties**, 457.
Niles, California, 121.
Nitrogen (sodium nitrate), used in nutrition experiments on *Nicotiana*, 283.
Nutrition, in relation to pollen germination, 280–281; in relation to sterility and flower-fall, 281–289; ferric chloride used in experiments in, 285.
Oxybathus, 350, 353.
Parthenogenesis, Parthenocarpy, and Phenospermy in *Nicotiana*, 249-271, 274–275.
Partial Sterility of *Nicotiana* **Hybrids Made with** *N. sylvestris* **as a Parent**, 189, 273, 293.
Pavia, 351.
Pelargonium, genus, 357.
Pedicel, histology of, 350; technique of examination, 361; increase in size and development of mechanical tissues, 369–371; induction of abscission in, 399–404, 414; throwing off of, 410; figures showing, opp. 420, 422, 426, 428.
Persian tobacco, 22.
Peru, 17, 19.
Petum angustifolium, 9.
Petunia, 367.
hybrida, 366, 398, 399, 411.
Petunioides, 19–30.
Phenospermy, application of term, 265, 269.
Phenotype variation, 490, 493.
Phosphorus used in nutrition experiments on *Nicotiana*, 283.
Physiological ''unit characters,'' 177-181, 197.
Physiology of *Nicotiana* hybrids, problems of, 275, 297, 313–314.

Pisum, 176, 446.
Pollen germination, experiments on, 276–281, 288, 289, 304; figures showing, opp. 292.
Pollination, effect of, on abscission of corolla, 388, 417; on time between anthesis and flower-fall, 389; premature, 433; self, efforts to secure artificially, 433–434.
Pollination, Controlled, in *Nicotiana*, 429; method and technique of experiments, 430; results of, 431.
Populus, 350, 352.
Potassium, used in nutrition experiments on *Nicotiana*, 284.
Quantitative characters, inheritance of, 459, 513, 514–516.
Quantitative Studies of Inheritance in *Nicotiana* Hybrids, 87, 169, 223.
Races in *Nicotiana Tabacum*, 458–459, 491.
Reaction system contrasts in heredity, 301, 313.
Residual heredity in *Tabacum*, 515-516, 520.
Ricinus, 351.
Rigor, in relation to poisonous gases, 445.
Rustica, section, 11–12; types of, 12-19.
Salix, 350, 352.
Salpichrora, 367.
rhomboidea, 366, 398, 399, 411.
Salpiglossus, 367.
sinuata, 366, 398, 399, 411.
Salvia, 350, 352.
splendens, 444.
Schilolysis, 353.
Seeding character of *Nicotiana* hybrids, 193.
Segregation, in *Nicotiana* hybrids, 192, 196, 247, 312–313, 459–460, and *passim*.
Separation cells, definition of, 349; in *Nicotiana*, 352.
Separation layer, definition of, 349; position of, 350; origin of, 351, 368; cytology of, 352; predetermined location of, 415.
Separation zone, definition of, 349; development of, 367–369; figure showing, 368.
Sessilifolia, derivatives, 475, 480, 485; × *macrophylla*, 487; × *auriculata*, 489.
Setchell, W. A., 1, 457.
Soil conditions, factor in abscission, 416.
Solanaceae, Abscission of Flowers and Fruits in, 347, 359.
Solanum jasminioides, 366, 398, 411.
marginatum, 366.
nigrum, 352, 366, 398, 399.
tuberosum, 350, 351, 366, 367, 411, 412.
umbelliferum, 366, 398, 411.
verbascifolium, 366, 371, 398, 399, 411.
Sprengel, cited, 19.
Stenophylla, derivatives, 474, 475–476, 484, 485; × *auriculata*, 490.
Sterility, Partial, of *Nicotiana* **Hybrids Made with** *N. sylvestris* **as a Parent**, 189, 193, 273.
Style, abscission of, in *Datura*, and in *Nicotiana*, 384–385, 413; effect of removal of, on abscission, and on time of flower-fall of corolla, 388–389.
Sugar, influence of amount of, during abscission, 381.
Sulfuric acid treatment in germination of tobacco seed, 199, 200, 203, 209, 219, 220.
Taxonomic problems in *Nicotiana Tabacum*, 461.
Temperature, influence on abscission time, 397, 409, 414, 416; contributing factor in germination of tobacco seed, 451.
Teratological forms in *Tabacum*, 513.
Thomas, Mrs. R. H., production of parthenogenetic seed in *Nicotiana*, 249.
Tobacco smoke. *See* Narcotic vapors.
Turgor. *See* Cell turgor.
Turkey, tobacco seeds from, 5.
"Unit character" conception in studies of heredity, 174–186; bearing of, on flower size question in *Nicotiana* hybrids, 175; physiological, 177–181, 197.
Vegetative reproduction in *Nicotiana*, 197.
Verbascum, genus, 357.
Viability of seed, 209–215, 220, 233, 240, 246, 263, 453, 454.
Vitis, 350.
White, O. E., cited, 521.
White tobacco, 7–8, 233; plate showing, opp. 40, 461.

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Page 340, plate 45. Fig. 1 should be fig. 2; fig. 2 should be fig. 1.

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BY
WILLIAM ALBERT SETCHELL

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THOMAS HARPER GOODSPEED

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QUANTITATIVE STUDIES OF INHERITANCE IN *NICOTIANA* HYBRIDS. II.

BY
THOMAS HARPER GOODSPEED

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Index, pp. 355-360.

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Index, pp. 397-400.

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THOMAS HARPER GOODSPEED

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QUANTITATIVE STUDIES OF INHERITANCE IN NICOTIANA HYBRIDS. III.

BY
THOMAS HARPER GOODSPEED

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    Index, pp. 355-360.

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Index, pp. 397-400.

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Index, pp. 389-397.

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THOMAS HARPER GOODSPEED

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Index, pp. 355-360.

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Index, pp. 397-400.

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Index, pp. 389-397.

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BY

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Index, pp. 355-360.

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Index, pp. 397-400.

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Index, pp. 389-397.

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Vol. 7. 1916-

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Vol. 5, No. 10, pp. 293-299 November 7, 1916

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Index, pp. 389-397.

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Vol. 6. 1914-

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Vol. 7. 1916-

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WITH SPECIAL REFERENCE TO THE CONCEPTION OF REACTION SYSTEM CONTRASTS IN HEREDITY

BY

T. H. GOODSPEED AND R. E. CLAUSEN

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Vol. 2.

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8, 9, 10, 11. (In one cover.) The Resistance of Certain Marine Algae to Changes in Osmotic Pressure and Temperature. The Rôle of Osmotic Pressure in Marine Plants. On the Importance of Physiologically Balanced Solutions for Plants. The Antitoxic Action of Potassium on Magnesium. By W. J. V. Osterhout. Pp. 227-236. March, 1906 ..... .25
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15. On Nutrient and Balanced Solutions, by W. J. V. Osterhout. Pp. 317-318. October, 1907 ..... .05
16. A Synopsis of the North American Godetias, by Willis Linn Jepson. Pp. 319-354; plate 29. December, 1907 ..... .40

Index, pp. 355-360.

Vol. 3. 1907-1909.

1. Compositae of Southern California, by Harvey Monroe Hall. Pp. 1-302; plates 1-3, with a map. December, 1907 ..... 3.00
2. The Origin, Structure, and Function of the Polar Caps in *Smilacina amplexicaulis* Nutt., by H. D. Densmore. Pp. 303-330; plates 4-8. December, 1908 ..... .35
3. 4. (In one cover.) The Value of Sodium to Plants by Reason of Its Protective Action. On the Effects of Certain Poisonous Gases on Plants. By W. J. V. Osterhout. Pp. 331-340. June, 1908 ..... .10
5. Contributions to the Knowledge of the California Species of Crustaceous Corallines. I. by Maurice Barstow Nichols. Pp. 341-348; plate 9. December, 1908 ..... .10

6. Contributions to the Knowledge of the California Species of Crustaceous Corallines. II. by Maurice Barstow Nichols. Pp. 349-370; plates 10-13. April, 1909 ..... .15
7. New Chlorophyceae from California, by Nathaniel Lyon Gardner. Pp. 371-375; plate 14. April, 1909 ..... .10
8. Plantae Mexicanae Purpusianae, I, by T. S. Brandegee. Pp. 377-396. May, 1909 ..... .15

Index, pp. 397-400.

Vol. 4. 1910-1912.

1. Studies in Ornamental Trees and Shrubs, by Harvey Monroe Hall. Pp. 1-74; plates 1-11; 15 text-figures. March, 1910 ..... .75
2. Gracilariophila, a New Parasite on *Gracilaria confervoides*, by Harriet L. Wilson. Pp. 75-84; plates 12-13. May, 1910 ..... .10
3. Plantae Mexicanae Purpusianae, II, by T. S. Brandegee. Pp. 85-95. May, 1910 ..... .10
4. Leuvenia, a New Genus of Flagellates, by N. L. Gardner. Pp. 97-106; plate 14. May, 1910 ..... .10
5. The Genus Sphaerosoma, by William Albert Setchell. Pp. 107-120; plate 15. May, 1910 ..... .15
6. Variations in Nuclear Extrusion Among the Fucaceae, by Nathaniel Lyon Gardner. Pp. 121-136; plates 16-17. August, 1910 ..... .15
7. The Nature of the Carpostomes in the Cystocarp of *Ahnfeldtia gigartinoides*, by Ada Sara McFadden. Pp. 137-142; plate 18. February, 1911 ..... .05
8. On a Colacodasya from Southern California, by Mabel Effie McFadden. Pp. 143-150; plate 19. February, 1911 ..... .05
9. Fructification of Macrocystis, by Edna Juanita Hoffman. Pp. 151-158; plate 20. February, 1911 ..... .05
10. *Erythrophyllum delesserioides* J. Ag., by Wilfred Charles Twiss. Pp. 159-176; plates 21-24. March, 1911 ..... .15
11. Plantae Mexicanae Purpusianae, III, by T. S. Brandegee. Pp. 177-194. July, 1911 ..... .15
12. New and Noteworthy Californian Plants, I, by Harvey Monroe Hall. Pp. 195-208. March, 1912 ..... .15
13. Die Hydrophyllaceen der Sierra Nevada, by August Brand. Pp. 209-227. March, 1912 ..... .20
14. Algae Novae et Minus Cognitae, I, by William Albert Setchell. Pp. 229-268; plates 25-31. May, 1912 ..... .40
15. Plantae Mexicanae Purpusianae, IV, by Townshend Stith Brandegee. Pp. 269-281. June, 1912 ..... .15
16. Comparative Development of the Cystocarps of *Antithamnion* and *Prionitis*, by Lyman Luther Daines. Pp. 283-302; plates 32-34. March, 1913 ..... .20
17. Fungus Galls on *Cystoseira* and *Halidrys*, by Lulu May Estee. Pp. 305-316; plate 35. March, 1913 ..... .10
18. New Fucaceae, by Nathaniel Lyon Gardner. Pp. 317-374; plates 36-53. April, 1913 ..... .75
19. Plantae Mexicanae Purpusianae, V, by Townshend Stith Brandegee. Pp. 375-388. June, 1913 ..... .15

Index, pp. 389-397.

Vol. 5. 1912-.

1. Studies in *Nicotiana*, I, by William Albert Setchell. Pp. 1-86. December, 1912 ..... 1.25
2. Quantitative Studies of Inheritance in *Nicotiana* Hybrids, I, by Thomas Harper Goodspeed. Pp. 87-168. December, 1912 ..... 1.00
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4. On the Partial Sterility of *Nicotiana* Hybrids made with *N. Sylvestris* as a Parent, by Thomas Harper Goodspeed. Pp. 189-198. March, 1913 ..... .10
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4. Notes on the Californian Species of *Trillium L.* IV. Teratological Variations of *Trillium sessile* var. *giganteum* H. & A., by Thomas Harper Goodspeed. Pp. 69-100, plates 11-17. January, 1917 ..... .30

UNIVERSITY OF CALIFORNIA PUBLICATIONS
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BY

JOHN N. KENDALL

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    Index, pp. 355-360.

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Index, pp. 397-400.

Vol. 4. 1910-1912.

1. Studies in Ornamental Trees and Shrubs, by Harvey Monroe Hall. Pp. 1-74; plates 1-11; 15 text-figures. March, 1910 ..... .75
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10. *Erythrophyllum delesserioides* J. Ag., by Wilfred Charles Twiss. Pp. 159-176; plates 21-24. March, 1911 ..... .15
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13. Die Hydrophyllaceen der Sierra Nevada, by August Brand. Pp. 209-227. March, 1912 ..... .20
14. Algae Novae et Minus Cognitae, I, by William Albert Setchell. Pp. 229-268; plates 25-31. May, 1912 ..... .40
15. Plantae Mexicanae Purpusianae, IV, by Townshend Stith Brandegee. Pp. 269-281. June, 1912 ..... .15
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19. Plantae Mexicanae Purpusianae, V, by Townshend Stith Brandegee. Pp. 375-388. June, 1913 ..... .15

Index, pp. 389-397.

Vol. 5. 1912-.

1. Studies in *Nicotiana*, I, by William Albert Setchell. Pp. 1-86. December, 1912 ..... 1.25
2. Quantitative Studies of Inheritance in *Nicotiana* Hybrids, I, by Thomas Harper Goodspeed. Pp. 87-168. December, 1912 ..... 1.00
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12. Abscission of Flowers and Fruits in the Solanaceae, with Special Reference to *Nicotiana*, by John N. Kendall. Pp. 347-428, 10 text figures, plates 49-53. March, 1918 ..... .85

Vol. 6. 1914-

1. Parasitic Florideae, I, by William Albert Setchell. Pp. 1-34, plates 1-6. April, 1914 ..... .35

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14. New Pacific Coast Marine Algae, I, by Nathaniel Lyon Gardner, Pp. 377-416, plates 31-35. June, 1917 ..... .40

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Vol. 7. 1916-

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CONTROLLED POLLINATION IN *NICOTIANA*

BY

THOMAS HARPER GOODSPEED AND PIRIE DAVIDSON

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    Index, pp. 355-360.

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Index, pp. 397-400.

Vol. 4. 1910-1912.

1. Studies in Ornamental Trees and Shrubs, by Harvey Monroe Hall. Pp. 1-74; plates 1-11; 15 text-figures. March, 1910 ..... .75
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Index, pp. 389-397.

Vol. 5. 1912-.

1. Studies in *Nicotiana*, I, by William Albert Setchell. Pp. 1-86. December, 1912 ..... 1.25
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AN APPARATUS FOR FLOWER MEASUREMENT

BY
T. H. GOODSPEED AND R. E. CLAUSEN

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BY
T. H. GOODSPEED, J. M. McGEE AND R. W. HODGSON

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13. Die Hydrophyllaceen der Sierra Nevada, by August Brand. Pp. 209-227. March, 1912 ..... .20
14. Algae Novae et Minus Cognitae, I, by William Albert Setchell. Pp. 229-268, plates 25-31. May, 1912 ..... .40
15. Plantae Mexicanae Purpusianae, IV, by Townshend Stith Brandegee. Pp. 269-281. June, 1912 ..... .15
16. Comparative Development of the Cystocarps of *Antithamnion* and *Prionitis*, by Lyman Luther Daines. Pp. 283-302, plates 32-34. March, 1913 ..... .20
17. Fungus Galls on *Cystoseira* and *Halidrys*, by Lulu May Estee. Pp. 305-316, plate 35. March, 1913 ..... .10
18. New Fucaceae, by Nathaniel Lyon Gardner. Pp. 317-374, plates 36-53. April, 1913 ..... .75
19. Plantae Mexicanae Purpusianae, V, by Townshend Stith Brandegee. Pp. 375-388. June, 1913 ..... .15

    Index, pp. 389-397.

Vol. 5. 1912-.

1. Studies in *Nicotiana*, I, by William Albert Setchell. Pp. 1-86. December, 1912 ..... 1.25
2. Quantitative Studies of Inheritance in *Nicotiana* Hybrids, I, by Thomas Harper Goodspeed. Pp. 87-168. December, 1912 ..... 1.00
3. Quantitative Studies of Inheritance in *Nicotiana* Hybrids, II, by Thomas Harper Goodspeed. Pp. 169-188. January, 1913 ..... .20
4. On the Partial Sterility of *Nicotiana* Hybrids made with *N. sylvestris* as a Parent, by Thomas Harper Goodspeed. Pp. 189-198. March, 1913 ..... .10
5. Notes on the Germination of Tobacco Seed, I, by Thomas Harper Goodspeed. Pp. 199-222. May, 1913 ..... .25
6. Quantitative Studies of Inheritance in *Nicotiana* Hybrids, III, by Thomas Harper Goodspeed. Pp. 223-231. April, 1915 ..... .10
7. Notes on the Germination of Tobacco Seed, II, by Thomas Harper Goodspeed. Pp. 233-248. June, 1915 ..... .15
8. Parthenogenesis, Parthenocarpy and Phenospermy in *Nicotiana*, by Thomas Harper Goodspeed. Pp. 249-272, plate 35. July, 1915 ..... .25
9. On the Partial Sterility of *Nicotiana* Hybrids made with *N. sylvestris* as a Parent, II, by T. H. Goodspeed and A. H. Ayres. Pp. 273-292, plate 36. October, 1916 ..... .20
10. On the Partial Sterility of *Nicotiana* Hybrids made with *N. sylvestris* as a Parent, III, An Account of the Mode of Floral Abscission in the F_1 Species Hybrids, by T. H. Goodspeed and J. N. Kendall. Pp. 293-299. November, 1916 ..... .05
11. The Nature of the F_1 Species Hybrids between *Nicotiana sylvestris* and Varieties of *Nicotiana Tabacum*, with Special Reference to the Conception of Reaction System Contrasts in Heredity, by T. H. Goodspeed and R. E. Clausen. Pp. 301-346, plates 37-48. January, 1917 ..... .45
12. Abscission of Flowers and Fruits in the Solanaceae, with Special Reference to *Nicotiana*, by John N. Kendall. Pp. 347-428, 10 text figures, plates 49-53. March, 1918 ..... .85
13. Controlled Pollination in *Nicotiana*, by Thomas Harper Goodspeed and Pirie Davidson. Pp. 429-434. August, 1918 ..... .10
14. An Apparatus for Flower Measurement, by T. H. Goodspeed and R. E. Clausen. Pp. 435–437, plate 54, 1 figure in text. September 25, 1918 .05
15. Note on the Effects of Illuminating Gas and its Constituents in Causing Abscission of Flowers in *Nicotiana* and *Citrus*, by T. H. Goodspeed, J. M. McGee and R. W. Hodgson. Pp. 439-450. December, 1918 ..... .15

Vol. 6. 1914-.

1. Parasitic Florideae, by William Albert Setchell. Pp. 1-34, plates 1-6. April, 1914 ..... .35
2. *Phytomorula regularis*, a Symmetrical Protophyte Related to *Coelastrum*, by Charles Atwood Kofoid. Pp. 35-40, plate 7. April, 1914 ..... .05
3. Variation in *Oenothera ovata*, by Katherine Layne Brandegee. Pp. 41-50, plates 8-9. June, 1914 ..... .10
4. Plantae Mexicanae Purpusianae, VI, by Townshend Stith Brandegee. Pp. 51-77. July, 1914 ..... .25
5. The *Scinaia* Assemblage, by William Albert Setchell. Pp. 79-152, plates 10-12. October, 1914 ..... .75
6. Notes on Pacific Coast Algae, I, *Pylaiella Postelsiae*, n. sp., a New Type in the Genus *Pylaiella*, by Carl Skottsberg. Pp. 153-164, plates 17-19. May, 1915 ..... .15
7. New and Noteworthy Californian Plants, II, by Harvey Monroe Hall. Pp. 165-176, plate 20. October, 1915 ..... .15
8. Plantae Mexicanae Purpusianae, VII, by Townshend Stith Brandegee. Pp. 177-197. October, 1915 ..... .25
9. Floral Relations Among the Galapagos Islands, by A. L. Kroeber. Pp. 199-220. March, 1916 ..... .20

10. The Comparative Histology of Certain Californian Boletaceae, by Harry S. Yates. Pp. 221-274, plates 21-25. February, 1916 ..... .50
11. A Revision of the Tuberales of California, by Helen Margaret Gilkey. Pp. 275-356, plates 26-30. March, 1916 ..... .80
12. Species Novae vel Minus Cognitae, by T. S. Brandegee. Pp. 357-361. May, 1916 ..... .05
13. Plantae Mexicanae Purpusianae, VIII, by Townshend Stith Brandegee. Pp. 363-375. March, 1917 ..... .15
14. New Pacific Coast Marine Algae, I, by Nathaniel Lyon Gardner. Pp. 377-416, plates 31-35. June, 1917 ..... .40
15. An Account of the Mode of Foliar Abscission in *Citrus*, by Robert W. Hodgson. Pp. 417-428, 3 text figures. February, 1918 ..... .10
16. New Pacific Coast Marine Algae, II, by Nathaniel Lyon Gardner. Pp. 429-454, plates 36-37. July, 1918 ..... .25
17. New Pacific Coast Marine Algae, III, by Nathaniel Lyon Gardner. Pp. 455-486, plates 38-41. December, 1918 ..... .35

Vol. 7. 1916-.

1. Notes on the Californian Species of *Trillium* L. I, A Report of the General Results of Field and Garden Studies, 1911-1916, by Thomas Harper Goodspeed and Robert Percy Brandt. Pp. 1-24, plates 1-4. October, 1916 ..... .25
2. Notes on the Californian Species of *Trillium* L. II, The Nature and Occurrence of Undeveloped Flowers, by Thomas Harper Goodspeed and Robert Percy Brandt. Pp. 25-38, plates 5-6. October, 1916 ..... .15
3. Notes on the Californian Species of *Trillium* L. III, Seasonal Changes in *Trillium* Species with Special Reference to the Reproductive Tissues, by Robert Percy Brandt. Pp. 39-68, plates 7-10. December, 1916 ..... .30
4. Notes on the Californian Species of *Trillium* L. IV, Teratological Variations of *Trillium sessile* var. *giganteum* H. & A., by Thomas Harper Goodspeed. Pp. 69-100, plates 11-17. January, 1917 ..... .30

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NOTE ON THE RELATION OF LIGHT AND DARKNESS TO GERMINATION

BY
T. HARPER GOODSPEED

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11. Plantae Mexicanae Purpusianae, III, by T. S. Brandegee. Pp. 177-194. July, 1911 ..... .15
12. New and Noteworthy California Plants, I, by Harvey Monroe Hall. Pp. 195-208. March, 1912 ..... .15
13. Die Hydrophyllaceen der Sierra Nevada, by August Brand. Pp. 209-227. March, 1912 ..... .20
14. Algae Novae et Minus Cognitae, I, by William Albert Setchell. Pp. 229-268, plates 25-31. May, 1912 ..... .40
15. Plantae Mexicanae Purpusianae, IV, by Townshend Stith Brandegee. Pp. 269-281. June, 1912 ..... .15
16. Comparative Development of the Cystocarps of *Antithamnion* and *Prionitis*, by Lyman Luther Daines. Pp. 283-302, plates 32-34. March, 1913 ..... .20
17. Fungus Galls on *Cystoseira* and *Halidrys*, by Lulu May Estee. Pp. 305-316, plate 35. March, 1913 ..... .10
18. New Fucaceae, by Nathaniel Lyon Gardner. Pp. 317-374, plates 36-53. April, 1913 ..... .75
19. Plantae Mexicanae Purpusianae, V, by Townshend Stith Brandegee. Pp. 375-388. June, 1913 ..... .15

    Index, pp. 389-397.

Vol. 5. 1912-.

1. Studies in *Nicotiana*, I, by William Albert Setchell. Pp. 1-86. December, 1912 ..... 1.25
2. Quantitative Studies of Inheritance in *Nicotiana* Hybrids, I, by Thomas Harper Goodspeed. Pp. 87-168. December, 1912 ..... 1.00
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4. On the Partial Sterility of *Nicotiana* Hybrids made with *N. sylvestris* as a Parent, by Thomas Harper Goodspeed. Pp. 189-198. March, 1913 ..... .10
5. Notes on the Germination of Tobacco Seed, I, by Thomas Harper Goodspeed. Pp. 199-222. May, 1913 ..... .25
6. Quantitative Studies of Inheritance in *Nicotiana* Hybrids, III, by Thomas Harper Goodspeed. Pp. 223-231. April, 1915 ..... .10
7. Notes on the Germination of Tobacco Seed, II, by Thomas Harper Goodspeed. Pp. 233-248. June, 1915 ..... .15
8. Parthenogenesis, Parthenocarpy and Phenospermy in *Nicotiana*, by Thomas Harper Goodspeed. Pp. 249-272, plate 35. July, 1915 ..... .25
9. On the Partial Sterility of *Nicotiana* Hybrids made with *N. sylvestris* as a Parent, II, by T. H. Goodspeed and A. H. Ayres. Pp. 273-292, plate 36. October, 1916 ..... .20
10. On the Partial Sterility of *Nicotiana* Hybrids made with *N. sylvestris* as a Parent, III, An Account of the Mode of Floral Abscission in the F_1 Species Hybrids, by T. H. Goodspeed and J. N. Kendall. Pp. 293-299. November, 1916 ..... .05
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12. Abscission of Flowers and Fruits in the Solanaceae, with Special Reference to *Nicotiana*, by John N. Kendall. Pp. 347-428, 10 text figures, plates 49-53. March, 1918 ..... .85
13. Controlled Pollination in *Nicotiana*, by Thomas Harper Goodspeed and Pirie Davidson. Pp. 429-434. August, 1918 ..... .10
14. An Apparatus for Flower Measurement, by T. H. Goodspeed and R. E. Clausen. Pp. 435–437, plate 54, 1 figure in text. September 25, 1918 .05
15. Note on the Effects of Illuminating Gas and its Constituents in Causing Abscission of Flowers in *Nicotiana* and *Citrus*, by T. H. Goodspeed, J. M. McGee and R. W. Hodgson. Pp. 439-450. December, 1918 ..... .15
16. Notes on the Germination of Tobacco Seed, III. Note on the Relation of Light and Darkness to Germination, by T. Harper Goodspeed. Pp. 451-455. April, 1919 ..... .05

Vol. 6. 1914-.

1. Parasitic Florideae, by William Albert Setchell. Pp. 1-34, plates 1-6. April, 1914 ..... .35
2. *Phytomorula regularis*, a Symmetrical Protophyte Related to *Coelastrum*, by Charles Atwood Kofoid. Pp. 35-40, plate 7. April, 1914 ..... .05
3. Variation in *Oenothera ovata*, by Katherine Layne Brandegee. Pp. 41-50, plates 8-9. June, 1914 ..... .10
4. Plantae Mexicanae Purpusianae, VI, by Townshend Stith Brandegee. Pp. 51-77. July, 1914 ..... .25
5. The *Scinaia* Assemblage, by William Albert Setchell. Pp. 79-152, plates 10-12. October, 1914 ..... .75
6. Notes on Pacific Coast Algae, I, *Pylaiella Postelsiae*, n. sp., a New Type in the Genus *Pylaiella*, by Carl Skottsberg. Pp. 153-164, plates 17-19. May, 1915 ..... .15
7. New and Noteworthy Californian Plants, II, by Harvey Monroe Hall. Pp. 165-176, plate 20. October, 1915 ..... .15
8. Plantae Mexicanae Purpusianae, VII, by Townshend Stith Brandegee. Pp. 177-197. October, 1915 ..... .25

9. Floral Relations Among the Galapagos Islands, by A. L. Kroeber. Pp. 199-220. March, 1916 ..... .20
10. The Comparative Histology of Certain Californian Boletaceae, by Harry S. Yates. Pp. 221-274, plates 21-25. February, 1916 ..... .50
11. A Revision of the Tuberales of California, by Helen Margaret Gilkey. Pp. 275-356, plates 26-30. March, 1916 ..... .80
12. Species Novae vel Minus Cognitae, by T. S. Brandegee. Pp. 357-361. May, 1916 ..... .05
13. Plantae Mexicanae Purpusianae, VIII, by Townshend Stith Brandegee. Pp. 363-375. March, 1917 ..... .15
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15. An Account of the Mode of Foliar Abscission in *Citrus*, by Robert W. Hodgson. Pp. 417-428, 3 text figures. February, 1918 ..... .10
16. New Pacific Coast Marine Algae, II, by Nathaniel Lyon Gardner. Pp. 429-454, plates 36-37. July, 1918 ..... .25
17. New Pacific Coast Marine Algae, III, by Nathaniel Lyon Gardner. Pp. 455-486, plates 38-41. December, 1918 ..... .35
18. New Pacific Coast Marine Algae IV, by Nathaniel Lyon Gardner. Pp. 487-496, plate 42. January, 1919 ..... .15

Vol. 7. 1916-.

1. Notes on the Californian Species of *Trillium* L. I, A Report of the General Results of Field and Garden Studies, 1911-1916, by Thomas Harper Goodspeed and Robert Percy Brandt. Pp. 1-24, plates 1-4. October, 1916 ..... .25
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3. Notes on the Californian Species of *Trillium* L. III, Seasonal Changes in *Trillium* Species with Special Reference to the Reproductive Tissues, by Robert Percy Brandt. Pp. 39-68, plates 7-10. December, 1916 ..... .30
4. Notes on the Californian Species of *Trillium* L. IV, Teratological Variations of *Trillium sessile* var. *giganteum* H. & A., by Thomas Harper Goodspeed. Pp. 69-100, plates 11-17. January, 1917 ..... .30

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INHERITANCE IN NICOTIANA TABACUM

I

A REPORT ON THE RESULTS OF CROSSING CERTAIN VARIETIES

BY

WILLIAM ALBERT SETCHELL
THOMAS HARPER GOODSPEED
AND
ROY ELWOOD CLAUSEN

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Vol. 5. 1912–1922.

1. **Studies in *Nicotiana*. I, by William Albert Setchell. Pp. 1-86. December, 1912 ..... $1.25**
2. **Quantitative Studies of Inheritance in *Nicotiana* Hybrids. I, by Thomas Harper Goodspeed. Pp. 87-168, plates 1-28. December, 1912 ..... 1.00**
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6. **Quantitative Studies of Inheritance in *Nicotiana* Hybrids. III, by Thomas Harper Goodspeed. Pp. 223-231. April, 1915 ..... .10**
7. **Notes on the Germination of Tobacco Seed. II, by Thomas Harper Goodspeed. Pp. 233-248. June, 1915 ..... .15**
8. **Parthenogenesis, Parthenocarpy and Phenospermy in *Nicotiana*, by Thomas Harper Goodspeed. Pp. 249-272, plate 35. July, 1915 ..... .25**
9. **On the Partial Sterility of *Nicotiana Hybrids* made with *N. sylvestris* as a Parent. II, by T. H. Goodspeed and A. H. Ayres. Pp. 273-292, plate 36. October, 1916 ..... .20**
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11. **The Nature of the F_1 Species Hybrids between *Nicotiana sylvestris* and Varieties of *Nicotiana Tabacum*, with Special Reference to the Conception of Reaction System Contrasts in Heredity, by T. H. Goodspeed and R. E. Clausen. Pp. 301-346, plates 37-48. January, 1917 ..... .45**
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13. **Controlled Pollination in *Nicotiana*, by Thomas Harper Goodspeed and Pirie Davidson. Pp. 429-434. August, 1918 ..... .10**
14. **An Apparatus for Flower Measurement, by T. H. Goodspeed and R. E. Clausen. Pp. 435-437, plate 54, 1 figure in text. September, 1918 ..... .05**
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Index in press.

Vol. 6. 1914–1919.

1. Parasitic Florideae, by William Albert Setchell. Pp. 1-34, plates 1-6. April, 1914 ..... .35

2. *Phytomorula regularis*, a Symmetrical Protophyte Related to *Coelastrum*, by Charles Atwood Kofoid. Pp. 35-40, plate 7. April, 1914 ..... .05

3. Variation in *Oenothera ovata*, by Katherine Layne Brandegee. Pp. 41-50, plates 8-9. June, 1914 ..... .10

4. Plantae Mexicanae Purpusianae. VI, by Townshend Stith Brandegee. Pp. 51-77. July, 1914 ..... .25

5. The *Scinaia* Assemblage, by William Albert Setchell. Pp. 79-152, plates 10-16. October, 1914 ..... .75

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7. New and Noteworthy Californian Plants. II, by Harvey Monroe Hall. Pp. 165-176, plate 20. October, 1915 ..... .15

8. Plantae Mexicanae Purpusianae. VII, by Townshend Stith Brandegee. Pp. 177-197. October, 1915 ..... .25

9. Floral Relations among the Galapagos Islands, by A. L. Kroeber. Pp. 199-220. March, 1916 ..... .20

10. The Comparative Histology of Certain Californian Boletaceae, by Harry S. Yates. Pp. 221-274, plates 21-25. February, 1916 ..... .50

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12. Species Novae vel Minus Cognitae, by T. S. Brandegee. Pp. 357-361. May, 1916 ..... .05

13. Plantae Mexicanae Purpusianae. VIII, by Townshend Stith Brandegee. Pp. 263-375. March, 1917 ..... .15

14. New Pacific Coast Marine Algae. I, by Nathaniel Lyon Gardner. Pp. 377-416, plates 31-35. June, 1917 ..... .40

15. An Account of the Mode of Foliar Abscission in *Citrus*, by Robert W. Hodgson. Pp. 417-428, 3 text figures. February, 1918 ..... .10

16. New Pacific Coast Marine Algae. II, by Nathaniel Lyon Gardner. Pp. 429-454, plates 36-37. July, 1918 ..... .25

17. New Pacific Coast Marine Algae. III, by Nathaniel Lyon Gardner. Pp. 455-486, plates 38-41. December, 1918 ..... .35

18. New Pacific Coast Marine Algae. IV, by Nathaniel Lyon Gardner. Pp. 487-496, plate 42. January, 1919 ..... .15

19. Plantae Mexicanae Purpusianae. IX, by Townshend Stith Brandegee. Pp. 497-504. November, 1919 ..... .05

Index in preparation.

Vol. 7. 1916–.

1. Notes on the Californian Species of *Trillium* L. I, A Report of the General Results of Field and Garden Studies, 1911-1916, by Thomas Harper Goodspeed and Robert Percy Brandt. Pp. 1-24, plates 1-4. October, 1916 ..... .25

2. Notes on the Californian Species of *Trillium* L. II, The Nature and Occurrence of Undeveloped Flowers, by Thomas Harper Goodspeed and Robert Percy Brandt. Pp. 25-38, plates 5-6. October, 1916 ..... .15

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